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"A STUDY CONCERNING THE DETERMINATION OF ORGANIC PHOSPHORUS IN SOILS"

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ABSTRACT

The different methods available for the determination of soil organic phosphorus are open to criticism. In the literature, the Kaila and Virtanen method (54) and the Mehta *et al*. method (67) in particular have been disputed as to which will yield the greater amount of organic phosphorus. The first objective of the present study was to evaluate these two extraction type methods by applying them to some Alberta soils. Subsequently, the study was expanded to determine whether a relationship existed between organic phosphorus and other soil properties including pH, total phosphorus, organic carbon, organic nitrogen and non-sulphate sulphur. The second objective was to evaluate an additional extraction type procedure (49) and two ignition type procedures (59, 84) which have been used by various workers for determining the total organic phosphorus content of soils. The third objective was to ascertain a workable modification of one or more of these methods particularly with the aid of the latest findings in the use of organic matter extractions.

Soils from six soil zones of Alberta were sampled in duplicate in the field. The data showed that there was no appreciable difference between the Kaila-Virtanen and Mehta *et al*. methods for the soils studied. Chemical and statistical analyses suggested that organic phosphorus formed an integral part of organic matter.

The ignition procedures appeared to be unsatisfactory as a result of incomplete combustion or of increased solubility of the original inorganic phosphorus. The Kaila and Virtanen method, which uses a mild extractant, seemed to be the best method for determining total organic phosphorus. However, by the addition of known phosphorus bearing compounds it was shown that all methods were very empirical indeed.

The Kaila and Virtanen method was improved by making modifications. However, it was reasoned that it was not possible to extract all the organic phosphorus present in a soil with one procedure. Efforts should be turned to obtain and evaluate not only definite forms of organic phosphorus, but also a better appreciation of the bonds between humic acids and phosphorus.

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I. INTRODUCTION

One of the most important differences between a parent material and a soil formed from it is the content of organic matter. The study of this organic component of soil is rather challenging. The six major elements in soil organic matter are carbon, hydrogen, oxygen, nitrogen, sulphur, and phosphorus. Of these phosphorus and sulphur normally come from the parent material. Walker (111) emphasized that the phosphorus content of parent materials will have an important influence on the quantity and nature of soil organic matter formed.

Phosphorus, after it has been absorbed from the soil, forms an integral part of many important compounds in plants and animals, such as nucleic acids, phytin, phospholipids, and sugarphosphates. It also plays a major role in many metabolic reactions. Subsequently, the phosphorus is returned to the soil mainly in organic combinations.

When the organic complexes contain little or no phosphorus, bacteria and fungi are able to remove from the soil some of the soluble phosphates, which are essential for cell synthesis (110). Thus, organic phosphorus in the soil may originate from organic residues added to the soil, and/or through synthesis by microorganisms. Organic phosphorus, therefore, represents that phosphorus which is present in the soil as a result of biological fixation of inorganic phosphorus by plants, animals, and microorganisms.

Organic phosphorus compounds, as with most organic materials, are not stable end products. For this reason the amount of organic phosphorus present in the soil varies considerably. The amount, in a sample taken at a particular instant, gives only an instantaneous image. It neither indicates its significance in the phosphorus cycle of the soil, nor measures the extent of conversion of organic phosphorus over a specific time interval.

Observations, such as lower organic phosphorus content in cultivated soils as compared with adjacent virgin areas (103), suggest that organic phosphorus is subject to decomposition and/or is utilized by plants. This concept is in contrast to the views of certain other workers (35), who regard organic phosphorus as an accumulation of compounds which are resistant to further decomposition. This accumulation gives rise then to a relatively unavailable fraction of the total soil phosphorus content. It has, therefore, been assumed that the importance of soil organic phosphorus with regard to its uptake by plants is nil. In view of these controversies more attention has been paid to the inorganic than the organic phosphorus fraction. It would, therefore, seem essential that more study be devoted to such aspects as the various forms of organic phosphorus in the soil.

Evaluations of the plant requirement for phosphorus depend, in large measure, upon a knowledge of the phosphorus status in the entire root zone. This root zone can extend considerably into the parent material. An analysis of the phosphorus status, both organic and inorganic, to the full depth of geochemical weathering is therefore necessary. Such an analysis may also help to elucidate the movement of organic phosphorus within the solum.

It has been assumed that the same compounds found in plants and microorganisms should be present in the soil. Isolation of decomposition products from soils has given strength to this assumption. However, as has been shown in one of the latest reviews on the subject (22), only a small fraction of the organic phosphorus of soil can be definitely accounted for and, consequently, the nature of the bulk of the organic phosphorus is still unknown.

Organic phosphorus compounds in the soil are difficult to isolate and identify. This is attributable to several causes, such as the variety of organic phosphorus compounds present in plants and microorganisms, and the relative ease with which many organic reactions occur. It was shown in 1940 (122) that, although part of the soil organic phosphorus bore a chemical similarity to nucleic acids, it differed greatly from such compounds in its susceptibility to decomposition in the soil. After 21 years our knowledge, with regard to individual organic phosphorus compounds, is still very deficient.

The different methods available for the determination of soil organic phosphorus are open to criticism. Nevertheless, the use of these methods has established beyond doubt the presence in soil of phosphorus in organic combinations. This fraction, in some cases, comprises more than half of the total soil phosphorus.

This investigation was undertaken--

(1) to evaluate two extraction-type methods, disputed in the literature as to which one would yield the greater amount of organic phosphorus, by applying them to some Alberta soils. Subsequently, it was thought of academic interest to relate the organic phosphorus in these soils to such other soil properties as pH, total phosphorus, organic carbon and nitrogen, and non-sulphate sulphur.

(2) to evaluate an additional extraction-type procedure and two ignition-type procedures which have been used by various workers for determining the total organic phosphorus content of soils.

(3) to evolve a workable modification of one or more of these methods particularly with the aid of the latest findings in the use of organic matter extractions.

II. REVIEW OF LITERATURE

"Das quellsatzsaure Kupferoxyd kann zur Analyse verwandt werden; jedoch sind die durch Kali aufgelösten Stoffe, welche mit Kupferoxyd unlösliche Verbindungen eingehen können, durch das essigsäure Kupferoxyd mit niedergeschlagen worden, hauptsächlich die Kiesel-und Phosphorsäure; diese stehen aber der Analyse des quellsatzsauren Kupferoxyds nicht im Wege."

Mulder: Ueber die Bestandtheile der Ackererde (1844; Translation in Appendix)

(A) Introduction

From the time that Mulder (71) indirectly implied the presence of organic phosphorus in the soil by observing that the organic matter of soils was not readily freed from phosphorus, until the turn of the century most work was of a qualitative nature. Schmoeger (87, 88) was probably one of the first to recognize the possible existence in soils of the organic compounds found in plants, animals, and microorganisms. Later, investigations concentrated on the proof of existence of organic phosphorus in soils by isolating from the soil definite phosphorus containing organic compounds, such as nucleic acids and phytins (e.g. 93). At present, methods to measure organic phosphorus quantitatively, both total and specific compounds, are being sought.

Many comprehensive reviews concerning organic phosphorus have been written (11, 13, 21, 22, 50, 77, 105). The review by Black and Goring in 1953 (13) in particular examines critically the history of organic phosphorus and gives an estimation of its quantity in soils. In spite of much research

only meagre information is available regarding the forms of organic phosphorus present in soils. Anderson (3, 4, 5, 6, 7) with the development of a series of procedures for the estimation of definite compounds has given a fresh impetus to this phase.

The distribution of organic phosphorus in the profile and the determination of total organic phosphorus will be discussed under separate headings. Organic phosphorus compounds are constituents of organic matter. A better understanding, therefore, of the agents used to extract organic matter in general may disclose new possibilities for the extraction of organic phosphorus in particular. Extraction of organic matter will be discussed under a third heading.

(B) Distribution of organic phosphorus in the profile

Most of the studies concerning the vertical distribution of phosphorus in soils have been concerned with total or inorganic phosphorus. Only a limited number of investigations reported deal with the vertical distribution of organic phosphorus. A direct comparison of the data published over the last 50 years is almost impossible due to the evolution of methods that have been used for determining total organic phosphorus.

The following review aims to evaluate some of the more pertinent contributions which deal with the whole solum. A true evaluation of the organic phosphorus content of soils must be based upon the whole solum and not just the surface

layer. Special attention is given to other constituents in the soil, such as organic nitrogen and carbon, and sulphur. Qualitative differences of organic phosphorus in various soils from the same and different regions are indicated.

In general, the amounts of organic phosphorus in soils are correlated positively with the amounts of organic carbon and nitrogen. Black and Goring (13) summarized the results of many workers and reported a mean C:N:organic P ratio in soils of 110:9:1 by weight. This ratio is wider for organic soils. It also appeared that the ratio increased with pH. They assumed that the rate of accumulation of organic phosphorus per unit of carbon and nitrogen was similar in acid and alkaline soils, but the resistance to mineralization was greater in acid than in alkaline soils. Walker et al. (114), after stating that sulphur as well as phosphorus were definite constituents of soil organic matter, suggested that soil organic matter may have a C:N:S:organic P ratio of 100:10:1:1. Some of the many organic carbon, nitrogen and phosphorus, and non-sulphate sulphur ratios cited in the literature are presented in Table 1.

The earliest work with regard to the vertical distribution of organic phosphorus was conducted by Schollenberger (91, 92). He sampled profiles in six-inch layers to a depth of 24 inches. The reaction of the soil appeared to have no influence upon the quantity and nature of the organic phosphorus present. Although the organic phosphorus was closely related to the total nitrogen in the soil, there did not appear

TABLE I. SOME OF THE MANY ORGANIC CARBON, NITROGEN, AND PHOSPHORUS; AND NON-SULPHATE SULPHUR RATIOS CITED IN THE LITERATURE

Ratio		Worker
C : N : S	C : N : P	
135 : 10 : 1.1(podzolic soils)		Evans and Rost (36)
116 : 10 : 1.5(chernozemic soils)		ditto
120 : 10 : 1.3(to a depth of 21")		Walker and Adams (112)
140 : 10 : 1.0		Walker, Adams and Orchiston (114)
155 : 10 : 1.4		Williams and Donald (117)
152 : 10 : 1.2(alkaline soils)		Williams and Steinbergs (118)
140 : 10 : 1.5(acid soils)		ditto
140 : 10 : 1.4		Williams, Williams and Scott (119)
	110 : 9 : 1.0(122 : 10 : 1.1)	Black and Goring (13)
	216 : 10 : 1.4(volcanic upland soils)	Kosaka and Abe (57)
	167 : 10 : 0.6(non-volcanic soils)	ditto
	182 : 10 : 0.5(virgin soils)	ditto
	155 : 10 : 1.0(same soils,cultivated)	ditto
	108 : 9 : 1.0(120 : 10 : 1.1)	Martinez de Pancorbo and Lucena Conde (64)
	120 : 10 : 2.7(to a depth of 21")	Walker and Adams (112)
	155 : 10 : 0.7	Williams and Donald (117)
	152 : 10 : 0.7	Williams and Steinbergs (118)
	147 : 10 : 2.5(non-calcareous soils)	Williams, Williams and Scott (119)
	113 : 10 : 1.3(calcareous soils)	ditto

to be any connection with other soil constituents.

Different methods measure different amounts of organic phosphorus. This is well illustrated when comparing the work of Auten (10) with that of Pearson and Simonson (76) for similar Iowa soils. Auten's values for the total organic phosphorus contents of the upper horizons were much lower than the ones reported by Pearson and Simonson (76). Values for the subsoil, on the other hand, were much higher. The ratios of organic phosphorus to organic carbon and nitrogen were found to vary considerably within individual profiles, as well as from one soil to another. Smaller variations occurred in the nitrogen-phosphorus ratio than in the carbon-phosphorus ratio (76).

Odynsky (73) discussed the solubility and distribution of phosphorus in five Alberta soils, namely one Brown, two Black and two Grey Wooded profiles. The organic phosphorus varied amongst the different profiles both in amount and percentage of the total phosphorus. For the surface layer of these profiles the organic phosphorus contents were 349 p.p.m. (58 per cent) for the Brown A_1, 712 and 375 p.p.m. (76 and 43 per cent) for the Black A_1, and 732 and 1105 p.p.m. (65 and 64 per cent) for the Grey Wooded A_o. Most of the organic phosphorus in the profiles was found in the surface horizons where it generally constituted a much greater proportion of the total phosphorus than the easily soluble phosphorus (soluble in H_2SO_4 at pH 3.0 ± 0.2). In the lower horizons, the organic phosphorus constituted a relatively small proportion of the

total phosphorus most of which was easily soluble.

Kheifets (55) showed that a difference exists in the relationship of organic versus mineral phosphorus in different horizons. A particular sharp change was observed in strongly podzolized soils, while the change occurred more gradually in Grey Wooded and Black soils. Undoubtedly, different forms of organic phosphorus were present in the profiles with some forms being more stable than others. He also suggested that phytin types of compounds were extracted by 4N HCl, while nucleic acids dissolved in four per cent NH_4OH.

Fuller and McGeorge (41) observed that, for the most part, those soils having the largest amount of organic carbon had the largest amount of organic phosphorus. Even though a relationship exists between these two soil components, the proportion of organic carbon to organic phosphorus may differ with different soils. The ratio of organic carbon to organic phosphorus in Arizona soils, for example, was less than half the values reported for surface soils in Iowa (76), even though the Arizona soils contained only slightly less total organic phosphorus than those from Iowa. They concluded that organic carbon was a primary variable between these two groups of soils. Mineralization of the organic carbon in these calcareous Arizona soils may have been much faster than in the more acid Iowa soils. Comparison of these two groups of data was quite valid, because all were obtained by the same analytical method. On the whole, the Arizona soils were rather well supplied with organic phosphorus (42), although the amount

decreased rapidly with increase in depth. Below 24 inches the organic phosphorus comprised only a very small part of the total phosphorus present. The amounts found below the 24-inch layer in these Arizona soils were similar to those for Iowa soils (75, 76), but significantly higher than quantities reported for 30 virgin Oklahoma soils (43).

Soil formation will have a definite effect upon the organic phosphorus status of soils. The vertical distribution of phosphorus has been altered by organic matter accumulation and, very likely, by a downward movement of phosphorus-bearing materials in suspension or in true solution (47). Allaway and Rhoades (2) noted a shift of acid-soluble forms of phosphorus to organic phosphorus compounds particularly with soils which remained neutral or only slightly acid during soil formation. Quantity, form, and properties of the humus fraction, as well as its relation to the mineral fraction of the soil, largely determines the accumulation, stability, or decomposition of organic phosphorus in the different horizons (81). The content of total and organic phosphorus varies with the degree of weathering in the profile (47, 81, 113).

Walker and Adams (113) found that the percentages of carbon, nitrogen, sulphur, and organic phosphorus tended to increase in weakly weathered soils. In moderately weathered soils the organic phosphorus decreased markedly with increased leaching. In strongly weathered soils contents of all elements tended to decline. A striking de-

crease in total phosphorus was observed with increased leaching in all sequences.

In general, organic phosphorus and associated nitrogen reached a peak at intermediate stages of weathering, corresponding roughly with the grassland-forest transition zones. Carbon continued to accumulate for a time in forest soils, giving soils with high C:N ratios in the upper horizons. With increased rainfall in these transition zones plant growth improved, more organic matter accumulated, and organic phosphorus became a bigger fraction of the total phosphorus. As a result, wide variations in the C:N:S:organic P ratio were found (113).

After studying relationships between carbon, nitrogen, sulphur, and organic phosphorus of certain New Zealand soils, Walker and Adams (112) concluded that the major factor governing the accumulation of organic matter was the phosphorus content of the parent material. The average C:N:S:organic P ratio for two soils to a depth of 21 inches was 120:10:1.3:2.7. The N:organic P ratio varied between soils being high for soils low in phosphorus (e.g. 10:1.2) and low for soils high in phosphorus (e.g. 10:3.2). The ratio also narrowed with increased depth which substantiated data found by Saunders (82). Saunders (83) suggested, as an additional factor for accumulation, a stabilization of some of the organic phosphorus compounds by reaction with the active clay. Similarly, Jahn-Deesbach (52) found that organic material supplied to the soil contained less phosphorus than the soil organic matter. This enrichment of the soil organic matter with phosphorus could be

the result of fixed organic substances, such as nucleic acids on clay, or inositol hexaphosphates on iron and aluminum.

The C:organic P ratios of soils studied by Walker and Adams (112) varied in the 0-7 inch layer from 111:1, and in the 14-21 inch layer from 18:1 to 30:1. Generally, the C:organic P ratio narrowed more than any other ratio with increased depth in the profile. These ratios were comparable to those found by Jahn-Deesbach (52) in chernozems, being 45-96:1 and 19-55:1 in A and C horizons respectively.

Halstead et al. (48), working with Eastern Ontario soils, found the average C:organic P ratio for the surface samples to be 105 as compared with 61 for the samples taken at 14-21 inch depth. The average N:organic P ratio was 8.0 for the surface samples and 4.9 for the subsurface samples. On the other hand, the C:organic P and N:organic P ratios reported for the seven Iowa soils studied by Pearson and Simonson (76) were lower, in many instances, in the surface layers than in those at greater depth. However, this opposite behaviour may be more apparent than real due to different extraction procedures and different forms of organic phosphorus.

Various workers have reported that different organic phosphorus compounds may dominate in different profiles. Sauerlandt et al. (81) found the organic phosphorus of a chernozem (pH 6.3 - 7.1) to be very stable. It could neither

be extracted with water nor with 1N HCl, but more than 90 per cent was extracted with 25 per cent NH_4OH solution. This form of organic phosphorus was also resistant to microbial activity. In a podzol (pH 3.5 - 4.5), on the other hand, the highest amounts were found in the acid extracts, although all the organic phosphorus could only be educed by extracting twice with NH_4OH. The large amounts present in the A_o and B horizons (250 and 200 p.p.m. resp.) were most likely due to the unfavourable conditions for microbial activity rather than to the stability of the organic phosphorus. In both soils a definite relationship existed between organic carbon and organic phosphorus.

Williams and Steinbergs (118), working with some Australian soils, observed that for samples highest in nitrogen, i.e. nearest to the surface, the proportion of organic phosphorus to nitrogen was quite narrow. It appeared that this proportion increased with increasing depth not as a straight line but as a curvilinear relationship. Earlier work by Williams (116) substantiated this variation in the relative amounts of phosphorus in the soil organic matter.

Organic compounds containing phosphorus, such as inositol hexaphosphate and its lower esters, do not contain nitrogen or sulphur in the molecule. Even when part of the organic phosphorus is closely associated with the carbon, nitrogen, and sulphur in the organic matter, variations in the amounts of inositol hexaphosphates may result in low correlations of

organic phosphorus with total carbon, nitrogen, and sulphur. More detailed information on the nature and distribution of organic phosphorus compounds in different soils and their relationships with soil properties is necessary to clarify this aspect (118, 119).

The proportion of organic phosphorus to nitrogen may also increase with increasing depth as a straight line relationship depending upon the amounts of humic acids present in the different horizons. It has been said that phosphorus is one of the necessary elements in humic acids (38, 68). In fact, phosphates seem to play an important role in the colloidal equilibrium of the humic acids. Misterski and Loginov (68) divided the bonds between humic acids and the phosphorus into two types: a stable bond at low pH levels, which may be broken by the effect of strong acids and high temperatures, and a weak bond which dissociates at pH values below 4-5. The first bond, in their opinion, has the character of an ester similar to phosphorus proteins. Calcium ions seem to act as bridges connecting the phosphoric acid to the carboxyl groups in the humic acids. Different amounts of organic phosphorus may, therefore, be extracted depending upon the conditions of the extraction procedure, which, in turn, may result in different ratio behavior.

(C) Determination of total organic phosphorus

Only one general procedure has been in use for the determination of soil organic phosphorus (13). It determines inorganic phosphorus in extracts before and after some treatment to change the organic phosphorus to an inorganic form, the amount of organic phosphorus being obtained by difference. The treatment to change the organic phosphorus to the inorganic form may be applied to the soil itself (ignition type) or to an extract containing the organic phosphorus (extraction type).

Ignition type--The organic phosphorus is changed to the inorganic form in the soil. The difference in inorganic phosphorus extracted before and after the change is a measure of the organic phosphorus present in the soil. Several methods have been suggested to bring about this change, but at present the dry combustion or ignition type seems to have the widest application.

Extraction type--The soil is given an acid pretreatment followed by an alkaline extraction. The organic phosphorus is then regarded as the difference between the total-phosphorus and inorganic-phosphorus in the extracts. Both determinations are made colorimetrically. The inorganic-phosphorus is analyzed directly in the extract, while the total-phosphorus is measured after destruction of all the organic material present in the extract.

In spite of much discussion in the literature, no worker until recently (6), has been able to determine which of the two approaches is the more reliable. Also, many assumptions accompany these two approaches leading to many requirements, a variety of procedures, and often resulting in contradictory results.

One of the main shortcomings of this general procedure is that the values are obtained by difference, that is by indirect means. Normal variations and errors in sampling, extraction errors, and the type of instrument used for spectrophotometrically estimating the amounts present will add to or subtract from the organic phosphorus values. Although the data itself are subtracted, the absolute errors are additive. The per cent error can become large particularly in subsoil samples containing only small amounts of organic phosphorus. It has been correctly stated (6, 54) that organic phosphate differences of 20 p.p.m. or less between methods have little or no significance. That is, the amounts of organic phosphorus cannot be reported with greater precision than 10-20 p.p.m. of soil (54).

Saunders and Williams (84) and Williams (120) reported that poor agreement sometimes occurred between the two types of methods. This difference was thought to be a function of parent material and drainage conditions. Legg and Black (59) similarly stated that the relative values obtained by the two methods were not independent

of the nature of the soil as indicated by the significant interaction at the one per cent level between soils and methods. Therefore, it was suggested that the organic phosphorus content of soils should be determined by both approaches and reported as the mean of the obtained values.

It has been thought (21) that most of the organic phosphorus could be accounted for as phytin, nucleic acids, and their decomposition products. However, at present only a small fraction of the organic phosphorus of soil can be definitely accounted for as inositol phosphate and nucleic acid phosphorus (22, 61, 101). Thomas and Lynch (101), for example, separated the organic phosphorus extracted with HCl and NaOH treatments into three fractions using an anion-exchange column. The major proportion of the organic phosphorus was in the first fraction in an unidentified form. In the three fractions, meso-inositol hexaphosphate and a supposed isomer of this compound accounted for 2-12 per cent of the total organic phosphorus, while all three forms accounted for 55-65 per cent of the total organic phosphorus. There was indication with the aid of paper chromatography that some of the compounds in the first fraction were very complex. From this it can be concluded that the nature of the bulk of the organic phosphorus is still unknown.

The extraction procedure consists of an acid pre-treatment followed by one or more alkaline extractions, either at room temperature or at 90°, or 120°C (e.g. 54, 67). These steps are designed to remove all the organic

phosphorus without changing any of it to the inorganic form. The total inorganic phosphorus present in these extracts does not represent the total inorganic phosphorus present in the soil, but only that portion which is brought out by the extraction plus that derived from hydrolysis of organic phosphorus during extraction (13).

Mehta et al. (67) pointed out that the procedures in which organic phosphorus is extracted from the soil are the most useful for developmental purposes. For example, when organic phosphorus is extracted from soil and estimated from the increase in inorganic phosphorus upon decomposition of the organic compounds, there is little question that the results represent an estimate of organic phosphorus extracted. Nothing can be said, however, about the absolute accuracy of the method.

Pretreatment of the soil with an acid solution before alkaline extraction gives increased extraction of soil organic phosphorus. The acid removes exchangeable calcium and calcium carbonate. Calcium can precipitate from solution compounds which are soluble in alkaline solutions. The efficiency of "calcium-masking" reagents, such as oxalates and citrates, indicates that calcium is one of the chief interferents in the extraction of organic matter from soil (24). Saunders (83) and Williams (115) reported, however, that calcium removal is not the only factor involved. It is very probable that the pretreatment loosens the combination of the organic phosphorus with

mineral constituents, such as iron and aluminum, either by complexing action or by acid hydrolysis. Or, as Mehta et al. (67) speculated, the acid pretreatment would remove coatings of mineral material, such as iron oxide or clay. These coatings would be the reason that part of the soil organic phosphorus resisted extraction. Bohne (14) suggested a third advantage. The acid will dissolve the inorganic phosphorus to quite an extent and, consequently, the danger of adsorption of inorganic phosphorus onto the organic substances in the alkaline extract would be decreased considerably. As a result the two extracts should be analyzed separately.

The possibility of a relationship (60) between the effectiveness of an alkaline extract to remove organic phosphorus and the concentration of acid used in the pretreatment suggested that the role of the acid may be greater than merely the extraction of metallic cations. This was reasoned because the concentration of acid appeared to be an important factor. It was established that treatment with concentrated HCl at 70^{0}C for 10 minutes extracted 84 per cent of the total organic phosphorus from a particular soil studied. Using such strong acid extractants must result in some loss by hydrolysis. Anderson (6) found that certain organic phosphorus forms such as glucose-1-phosphate, were completely hydrolyzed in some soils even by mild acid treatment. He further showed that nucleic acids were

considerably hydrolyzed by strong acid pretreatments. Mild alkaline pre-pretreatment removed from soils several esters intact which would have been hydrolyzed by acid.

Treatment of soils with alkaline solutions results in a partial decomposition of the colloidal complex and large amounts of organic and mineral matter are brought into solution. All extractants with an alkaline reaction oxidize the material concurrently, producing carbon dioxide by oxidation of part of the organic matter and probably, altering it in other ways as well. The preparations thereby obtained are to some extent artifacts of the method (26). Of the different alkaline extractants available sodium hydroxide is at present one of the main reagents used in the extraction of total organic phosphorus (49, 54, 67, 84). Minerals of apatite structure have a low solubility in sodium hydroxide (121).

Another reason for the difficulty in extracting organic phosphorus is its formation of insoluble complexes with certain metallic cations, such as iron and aluminum. Ghani (45) found that some of the phosphorus brought into solution by an initial acetic acid treatment was readsorbed by iron and aluminum and then re-extracted by the subsequent alkaline treatment. This gave rise to low results for the acid extraction and high results for the alkaline extraction. He, therefore, suggested the use of 8-hydroxy quinoline as a means of blocking readsorption or precipitation of phosphate by active iron and aluminum during acid extraction which just extricated the phosphate from calcium. Williams (115),

exploring this suggestion somewhat further, showed an increase in the amount of organic phosphorus extracted from the use of this chemical. On the whole, compounds that are good polyvalent-metal extractants are also good organic matter extractants (25).

Saunders and Williams (84) found that adding 8-hydroxy quinoline to 2.5 per cent acetic acid (115) was equally as effective a pretreatment as acids such as 0.1N HCl. However, the 8-hydroxy quinoline, although complexing the iron and aluminum, interfered in the colorimetric determination of inorganic phosphorus and so prevented the determination of organic phosphorus that had been extracted by the acid pretreatment (69, 83). Bertheux (12), extracting his samples with acetic acid containing one per cent 8-hydroxy quinoline, freed the acid extract of the 8-hydroxy quinoline by evaporation with concentrated ammonia. Cooke (30), on the other hand, found in spite of the formation of insoluble complexes with iron and aluminum that 8-hydroxy quinoline reduced phosphate fixation by the oxides only slightly.

Boswall and DeLong (16), realizing that acid-alkaline extractions may cause hydrolysis and/or other chemical changes which would be particularly undesirable when studying the nature and identity of the compounds extracted, developed an extraction with 8-hydroxy quinoline in a water-benzene system at pH 9.2. This extraction followed a mild acid pretreatment for the release of organic phosphorus.

The proportion of the total organic phosphorus released into the aqueous phase approached that obtained by more drastic procedures. The main advantage, however, was that extraneous anions which might interfere with subsequent attempts to identify the phosphorus compounds extracted were not introduced.

In general, much more work has been done to improve the extraction type procedure than the ignition type, even though the latter is more rapid and simple. This lack of interest for improving the ignition methods is probably due to the lack of a suitable standard for comparison.

Saunders and Williams (84) found that organic phosphorus values by the ignition method are little affected by wide variations in the ignition temperature (400° - 650°C), concentration of acid (0.1N - 4N H_2SO_4), and period of extraction (2 - 16½ hours). At 800°C the organic phosphorus values were definitely low due to volatilization of phosphate. In fact, there was a general tendency for the ignition values to be slightly higher than those obtained by extraction procedures.

Organic matter decomposes gradually at 300° - 400°C. Odynsky (73) reasoned that more complete decomposition of the organic matter should take place at 600°C, particularly when it was held at this temperature for one hour, although no work was done to satisfactorily prove this viewpoint. Besides, at this temperature both aluminum--and iron-oxide

would be dehydrated to a considerable extent resulting in greatly reduced fixation power for phosphate.

Legg and Black (59) selected a temperature of 240°C. Ignition at high temperatures led to high values for organic phosphorus because of an increase in solubility of inorganic phosphorus. Ignition at low temperatures led to low values due to incomplete combustion of organic matter. It was found that at an ignition temperature of approximately 240°C the positive and negative errors were equal. They also raised a definite objection against using any extraction procedure as a standard. It was found that the increase in solubility of inorganic phosphorus during ignition was associated with the content of organic phosphorus and not with the content of acid-soluble inorganic phosphorus. This so-called increase in solubility of inorganic phosphorus could be, at least in part, the result of decomposition of organic phosphorus not extracted by the extraction procedure.

During the last ten years comparisons of methods have given conflicting results (6, 54, 59, 106, 119). Mehta et al. (67), comparing different extraction methods, regarded the method that gave the highest value as being the best. This is justified with alkaline extraction methods, because the two main sources of error being hydrolysis and incomplete extraction both resulting in low values. With ignition-acid extraction methods there is not the same certainty due to possible increases in the solubility of

the inorganic phosphorus during ignition. In general, the organic phosphorus values by the ignition methods have been higher than those by alkaline extraction (54, 59, 84, 119). However, lack of a reliable criterion prevents a clear decision in contradictory cases.

One of the more logical approaches has been taken by Anderson (6). Much evidence suggests that there are distinct qualitative differences in the organic phosphorus compounds present in various soil types of different regions and in various horizons. Alternately, the degree of success with which a procedure enables a given organic phosphorus compound to be estimated may depend upon the nature of the soil. The reliability of any given method could be better assessed if more information was available about the nature of the different forms of organic phosphorus present in soil.

(D) Extraction of organic matter

Bremner and Lees (24) stated that "the most serious difficulty encountered in the study of soil organic matter is that of separating the organic complexes from the inorganic material of the soil." At present no solvent which will dissolve the organic matter complex completely and unchanged is known. The problem concerning the isolation of organic substances from the mineral components of soil without chemically changing these substances starts with the extractant. Sodium hydroxide, for example, is used

extensively. However, it causes a partial decomposition of the colloidal complex resulting in considerable amounts of organic and mineral matter coming into solution.

One of the major objections to the use of sodium hydroxide as a solvent, or for that matter any extractant with an alkaline reaction, is the alteration of the material by hydrolysis and autoxidation (20). This is of course a definite disadvantage when determining the total organic phosphorus content in the soil in that it changes this phosphorus into inorganic forms. If certain organic phosphorus compounds are stable, a drastic reagent such as sodium hydroxide is still valuable, particularly for quantitative purposes. These alterations are highly undesirable, however, for the analysis of many other organic compounds. This is most likely one of the main reasons for the elusiveness of organic phosphorus.

The literature concerning the extraction of organic matter is getting rather voluminous. A survey of the more pertinent references will be illuminating and inspiring, particularly when some of the extractants mentioned may be tried either for total organic phosphorus, or for the recovery of definite phosphorus containing organic compounds. The proportions of certain fractions of organic matter obtained appear to depend on the treatment applied, because the many side reactions give rise to artifacts. Evans (37), for example, showed that the amount of organic matter extracted tended to increase with increase in pH of the extractant

solution. The same was true for the humic acid fraction in the extracts. The amounts extracted also increased with time and temperature.

Aqueous salt solutions, such as neutral solutions of sodium pyrophosphate (24) and sodium fluoride (24, 37, 90, 94, 95), have been widely employed for the extraction of organic matter. Their efficiency may be due to their ability to chelate or form co-ordination complexes with calcium and the trivalent metal ions. It is these polyvalent metal cations that are responsible for rendering humus compounds insoluble. These mild solvents, however, generally dissolve no more than a quarter of the organic matter from soils.

The amount of organic matter extracted when a soil is treated with alkali will be increased if the soil is first leached with acid (20, 29). This led Broadbent (27) to suggest that the separation of organic matter from the inorganic material be accomplished by dissolving the latter. Hydrofluoric acid readily dissolves hydrated silicate minerals (23, 29, 78, 89) which are the most chemically reactive materials, and thereby would be expected to increase the efficiency of the separation of organic matter from the inorganic fraction.

Hydrofluoric acid is a weak acid with an ionization constant of 1.7×10^{-5}. Strong electrolytes such as nitric and hydrochloric acids are completely ionized in aqueous solutions. Even the second ionization constant of sulphuric

acid is 1.2×10^{-2}. It is thought, therefore, that no serious modifications of the soil organic matter takes place from treating with hydrofluoric acid. Treatment of soils with 1N HF : 1N HCl solution at room temperature has little effect on the extraction of organic matter by neutral and alkaline reagents, but it releases considerable amounts of organic matter (23). Schnitzer and Wright (89) submitted two functions of the hydrofluoric acid treatment. One was the dissolution and complexing of iron and aluminum resulting in the liberation of carbon, and the other was the direct dissolution of some silicon thereby releasing the associated carbon.

Scheffer et al. (85) pointed out that extraction of weakly humified material with 0.5 per cent sodium hydroxide did not lead to the identification of low-molecular reactive components of the humic complex, since these compounds underwent drastic modifications. Their results suggested that where extraction with sodium hydroxide appeared unavoidable it should be preceded by extraction with organic solvents, and not vice versa, so as to obtain the low-molecular unstable humic substances.

The solvent properties of aqueous solutions of metal-chelating organic compounds such as 8-hydroxy quinoline, cupferron, and acetyl acetone were investigated by Martin and Reeve (62, 63). It was concluded that for organic matter to be extracted by solutions of organic chelate

compounds it must be immobilized by ions of one of the transition metals such as iron and aluminum, and that the hydrogen form must be readily dispersed. Acetyl acetone was the most useful reagent tested owing to its miscibility with water and the formation of stable ether-soluble complexes with iron and aluminum.

Parsons and Tinsley (74) pointed out that comparitively little attention has been given to non-aqueous organic liquids as solvents for soil organic matter. They found that undecomposed plant cellulose remained almost insoluble in anhydrous formic acid, but microbiological polysaccharides and protoplasmic components including proteins were soluble. The results showed, however, that more than half the organic matter remained undissolved. Soon after deposition and transformation the residual organic material was rendered insoluble by combination with metal ions or other inorganic components of the soil.

Particularly in Germany, many attempts have been made to distinguish the relatively undecomposed plant residues from the humified material by an acetylation procedure. Although the use of acetyl bromide as a reagent for distinguishing between these two groups of constituents of humus has been questioned (110), it does remove certain partially decomposed plant and microbial residues.

(E) Summary

In summary several general conclusions can be drawn from the work reviewed on organic phosphorus. In spite of the inaccuracies of the methods, it cannot be overlooked that a definite proportion of the phosphorus, particularly in the surface horizons, is held in organic combinations. The organic phosphorus content decreases with depth, the largest amounts occurring in the surface horizons. Organic phosphorus may give low correlations with organic nitrogen and carbon, and non-sulphate sulphur due to the presence of non-sulphur and non-nitrogen containing organic phosphorus compounds.

Different forms of organic phosphorus are present in different soils and in different horizons. Therefore, more detailed information is required about the nature of the different forms of organic phosphorus in soils. Also, some forms are more stable than others. Selective extraction will give low yields, but may give unchanged material for further examination.

The present day methods for determining organic phosphorus in soils are highly inadequate. Alkaline extractions may have to precede acid pretreatments. However, where extractions with sodium hydroxide appear unavoidable, this may have to be preceded again by extraction with organic solvents in order to obtain low-molecular, unstable substances. It is expected that treatment with hydrofluoric acid, particularly of the lower horizons, will increase the efficiency of the separation of organic matter.

III. MATERIALS AND METHODS

(A) Materials

The soils used for the two extraction-type procedures are typical of the different soil zones in Alberta. Soils representing these zones have been described in considerable detail (18, 72, 124, 125) and only brief descriptions need be given here. These soils all have medium textured, glacial till as their parent material. The legal locations are recorded in the Appendix and are shown in Figure 1.

The Brown, Dark Brown, Thin Black, Black, and Dark Grey soils are all placed in the Chernozemic Order. They developed under grassland vegetation in moderately to well drained locations and cover an area that ranges from semi-arid to sub-humid. The semi-arid Brown soil zone of South-Eastern Alberta has an average annual precipitation of about 12 inches, a high rate of evaporation, frequent hot, dry winds, and prolonged periods of drought. The A horizon is relatively shallow and brown in colour. Proceeding north and north-westward into the Dark Brown and Thin Black zones, the moisture efficiency increases mainly due to a decrease in the evaporation rate and less frequent hot winds. Under the more humid conditions of the Black soil zone the A horizon is deeper and of a darker colour than soils from the other zones. The division between the A and B horizons of these Chernozemic soils is usually

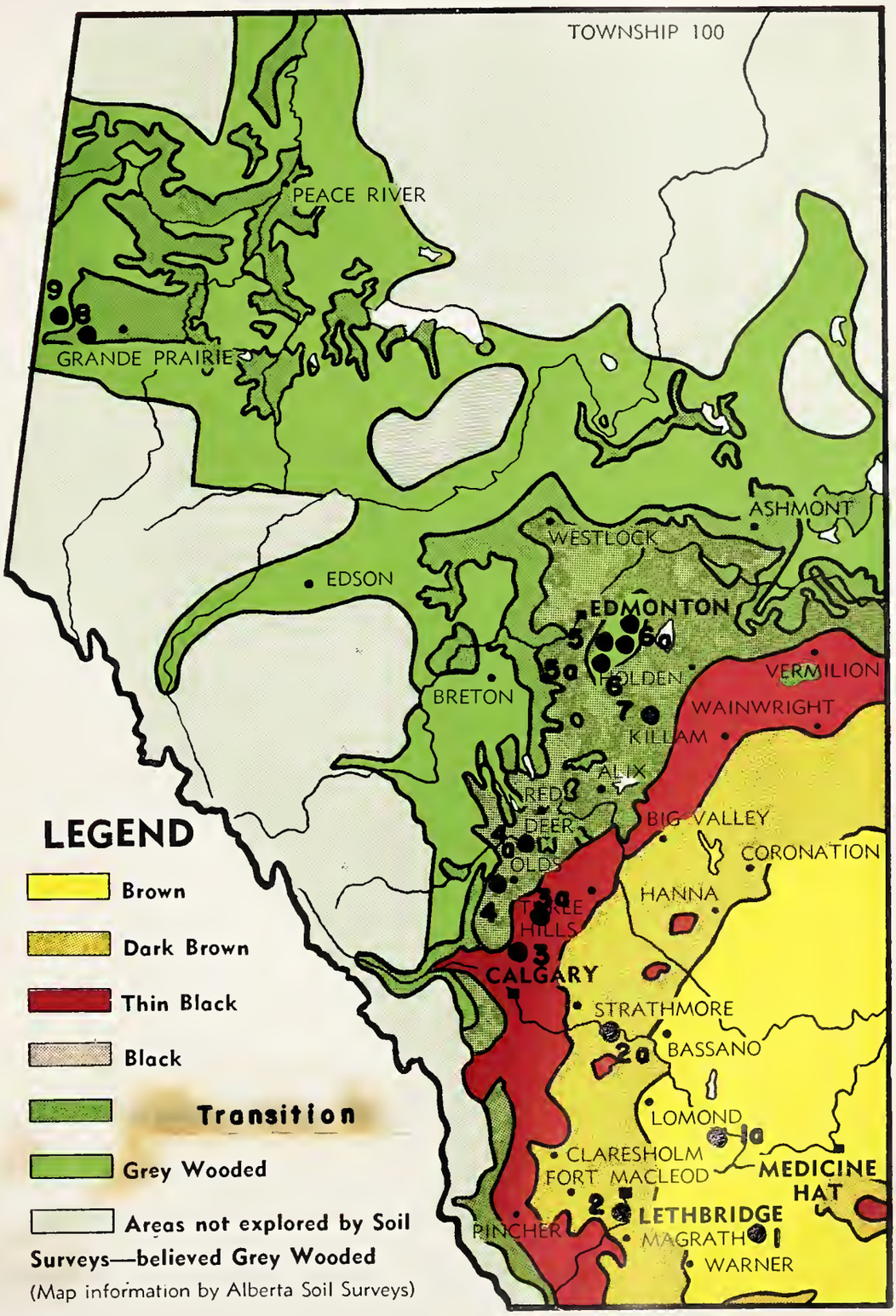

Figure 1. Locations of the profiles sampled.

gradual and indistinct. Soluble constituents, leached from the surface horizons, are not removed from the profile, but rather accumulate in a zone at a depth approximating the average penetration of the precipitation. As the precipitation increases, this zone of salt accumulation moves deeper into the profile. The Dark Grey soils form a part of the transition zone between the Chernozemic and Podzolic soils, that is the profile characteristics are intermediate between the Black and Grey Wooded soils. Leaching has taken place to a greater degree than for the Black and to a lesser degree than for the Grey Wooded soils. It seems likely (72) that a dark Ah horizon was formed under grass vegetation and that the leached layer, Ahe, was formed after encroachement of trees, because it is generally recognized that pronounced leaching is more apt to occur under trees than under grass. The calcium (lime) horizon has moved from near the surface down to a depth of 45 or 50 inches.

The Grey Wooded soil developed under a forest vegetation and is, therefore, relatively strongly leached giving rise to a distinct ashy Ae horizon. The C horizon usually contains lime.

The fate of the organic matter (53) in the more mature soils of Alberta should also briefly be mentioned. In virgin soils of the Chernozemic order the gradual

disintegration of the roots in a soil environment lacking sufficient oxygen will give rise to a considerable accumulation of humus. If this soil is high in lime this process will be accentuated. Organic matter is fixed by calcium as a result of its effective action as a coagulating agent of organic colloids.

In forest vegetation, the annual drop of leaves, needles, and other tree residues will bring about an extensive accumulation of organic matter on the surface of the soil. If these residues are not mixed with the underlying mineral layer through the activity of earthworms, insects, etc., this surface layer of humus may undergo considerable leaching. Certain organic and inorganic constituents will be removed from these upper horizons and reprecipitated in lower horizons. This series of events leads to the formation of Podzolic soils.

Plates I and II* show the profile characteristics of the six soils studied. Two profiles were taken from each soil giving a total of twelve profiles. Individual major horizons were sampled at virgin sites, placed in plastic bags, and taken to the laboratory. The samples were dried, passed through a 60-mesh sieve, and stored in quart sealers.

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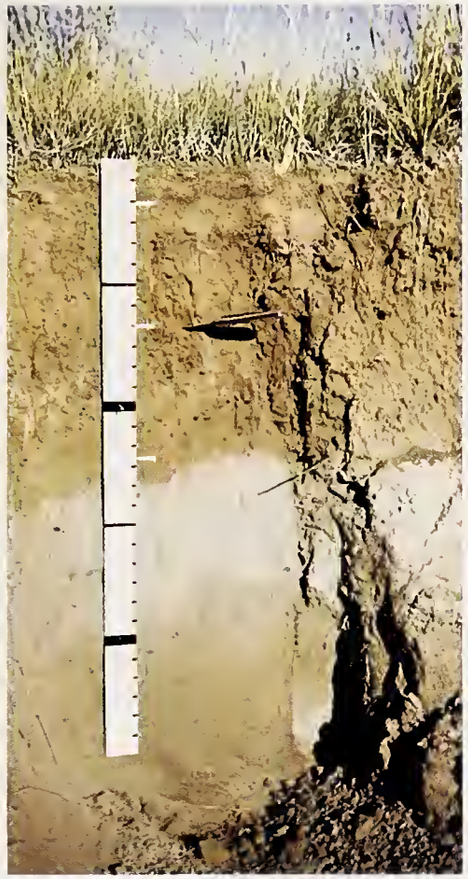
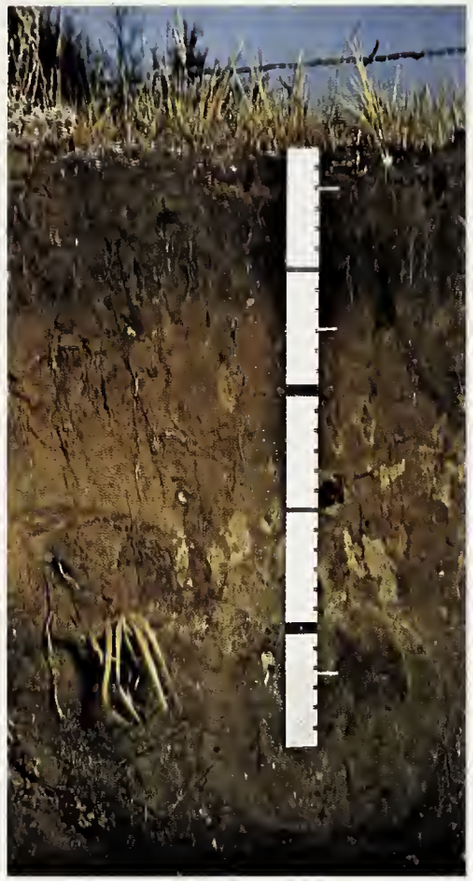
* The use of these plates is by courtesy of Mr. K. J. Spread, Research Officer, Alberta Assessment Commission, Department of Municipal Affairs, and Mr. D. A. Bancroft, Supervisor, Public Administration Training Division, Department of Extension, University of Alberta.



BROWN



DARK BROWN

THIN BLACK

Plate I. Profile characteristics of the Brown, Dark Brown, and Thin Black soils of Alberta.

BLACK DARK GREY GREY WOODED

Plate II. Profile characteristics of the Black, Dark Grey, and Grey Wooded soils of Alberta.

The soil used in the 'comparison of methods' and 'the recovery of added organic phosphorus' was one of the soils from McKeague's study (65). He identified it as profile 2 of site IV, a Black Solodized Solonetz from a gently undulating, till plain.

Finally, the peats used in the combustion trials were collected in Northern Alberta at Wembley for the sedge-peat and at Brainard for the moss-peat. Both peats are found in level to depressional areas with poor to very poor drainage. In the upper parts of the profile the sedge profile is dark-brown to brown in colour, and contains partially decomposed sedge and rush. The lower horizons consist of black, well-decomposed muck in which there are few recognizable leaf and stem remains. The moss-peat is composed of dark-brown to brown, undecomposed and partly decomposed Sphagnum-moss, tree roots, and stem remains in the upper parts of the profile. At greater depth the material is very dark brown to black and fairly well decomposed, but still contains recognizable stem and woody remains.

(B) Analytical Methods

The methods presented in this section are all standard procedures. Any special method or modification will be outlined under Results and Discussion. Solutions whose concentrations were determined by colorimetric measurements were read on a Coleman Universal Spectrophotometer, Model 14.

pH - A Beckman Model H-2 pH meter was used to measure the acidity of a saturated soilpaste.

Organic Carbon - Total carbon was determined by the manometric VanSlyke-Folch method (107) using the VanSlyke-Neill apparatus (108) and a combustion mixture consisting of H_3PO_4, fuming H_2SO_4 (20 per cent SO_3), KIO_3, and $K_2Cr_2O_7$ as described by VanSlyke *et al*. (109). Bremner (19) found this manometric method to be as accurate as the conventional dry-combustion procedure.

Inorganic carbon was determined by the same method, using the same apparatus, but the strong acid combustion mixture was substituted by 0.5N HCl containing 14 grams $FeCl_2.4H_2O$ per liter (19). Decarboxilation of uronide material was minimized by limiting the boiling period to 1½ minutes.

The difference between the total and inorganic carbon constituted the organic carbon content.

Organic Nitrogen - Total nitrogen was determined by the A.O.A.C. macro Kjeldahl procedure (9), except that a product (Kel-pak*) containing HgO(0.41 gm.), $CuSO_4$(0.08 gm.), and K_2SO_4(9.9 gm.) was used for the catalyst. The ammonia was collected in a four per cent H_3BO_3 solution as suggested by Meeker

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* D-C TAB No. 1, Digestion Catalyst Powders, Harshaw Chemical Co., Cleveland 6, Ohio.

and Wagner (66), and titrated with standard H_2SO_4.

Fixed ammonium was estimated by a procedure outlined by Stevenson and Dhariwal (100), using a micro-distillation apparatus as described by St. Arnaud (99).

The difference between the total nitrogen and fixed ammonium values constituted the organic nitrogen content.

Non-sulphate Sulphur - Total sulphur was determined by the method of Butters and Chenery (28), using the spectrophotometer at a wavelength of 490 mu. For both the total sulphur and total phosphorus analyses, the material was brought into solution by a wet-ashing procedure as outlined by Bohne *et al*. (15). A one-gram sample of soil was treated with five milliliters 70 per cent $HClO_4$ in a 50 ml. beaker, covered with a watch glass, and placed in a sand-bath. If the samples were high in organic matter, 13 ml. of concentrated HNO_3 were used in conjunction with the $HClO_4$ to predigest the material. When the HNO_3 was almost gone, the temperature was raised in order to bring the $HClO_4$ to a boil. The material was kept boiling for up to an hour after complete discolouration.

Inorganic sulphur in the form of gypsum was removed by digesting and washing the samples with hot 2N NH_4Cl*. The washed and dried material was then analyzed for total sulphur by the foregoing described procedure.

Total Phosphorus - The samples were first wet-ashed (15) and the filtrate made up to 100 ml. Total phosphorus was determined on a 10 ml. aliquot by a modification (44) of the molybdivanadophosphoric acid procedure as described by Kitson and Mellon (56). The spectrophotometer was set at a wavelength of 430 mu.

Total Organic Phosphorus - Five different methods were used in different phases of the work. These included the acid-alkaline extraction types by Mehta *et al*. (67), Kaila and Virtanen (54), and Hayashi and Takijima (49), and the ignition types by Legg and Black (59) and Saunders and Williams (84). A summary of the different steps taken with these five methods for total organic phosphorus is given in Table 2. Throughout the remainder of the thesis these methods will be referred to as Mehta *et al*., Kaila-Virtanen, etc. without making further reference each time to their number in the Bibliography.

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* Dr. S. Pawluk, Assistant Professor, Department of Soil Science, University of Alberta. Personal communication.

TABLE 2 SUMMARY OF THE STEPS OF THE FIVE METHODS USED FOR TOTAL ORGANIC PHOSPHORUS DETERMINATIONS

	Acid-alkaline extractions			Ignition	
Item compared	Hayashi-Takijima	Kaila-Virtanen	Mehta *et al.*	Legg-Black	Saunders-Williams
Acid pretreatment	Soil leached with 100 ml. 1% HCl or until free of Ca.	25ml.4N H_2SO_4 at room temp. for 18 hrs. Filter, wash.	10ml.conc.HCl at 70° for 10 min.Add 10ml. conc. HCl. Leave at room temp.for 1 hr. Centrifuge.		
Alkaline extraction -cold	10ml.1%NaOH at 60°C for 1 hr.Centrifuge.	100ml.0.5N NaOH at room temp.for 18 hrs.Centrifuge.	30ml.0.5N NaOH at room temp. for 1 hr. Centrifuge.		
-hot	10ml.2% NaOH at 90°C for 2 hrs. Centrifuge.	100ml.0.5N NaOH at 90°C for 4 hrs.Centrifuge.	60ml.0.5N NaOH at 90°C for 8 hrs.Centrifuge.		
Treatment of extracts	Combine all three extracts in 250 ml.flask. Take aliquots for total and inorganic P.	Combine alkaline extracts. Combine aliquots of acid and alkaline extracts for total and inorganic P.	Combine all three extracts in 250ml.flask. Take aliquots for total and inorganic P.		
Ignition temperature				240°C for 1 hr.	550°C for 1 hr.
Extraction of ignited and unignited sample				10ml.conc.HCl at 70°C for 10 min.Add 10ml. conc.HCl.Leave at room temp. for 1hr.Centrifuge.Fill up to 250 ml.Take aliquots.	100 ml.0.2N H_2SO_4 at room temp. overnight in end-over-end shaker. Filter.Fill up to 200ml.Take aliquots.

Inorganic and Total Phosphorus Contents of the Organic Phosphorus Extracts - Inorganic and total phosphorus were determined in each extract. Total phosphorus represented all phosphorus present in the extract and not the total phosphorus present in the soil. The difference between the total and inorganic phosphorus was regarded as the total organic phosphorus.

The colorimetric molybdenum blue method is specific for inorganic orthophosphate. As Black and Goring (13) pointed out, this method permits a clear differentiation between organic and inorganic phosphorus, providing the former is not subject to hydrolysis and the latter is present as soluble orthophosphate. Phosphorus which is bound to organic substances does not form a complex with MoO_3 and for this reason cannot be determined by the blue colour method. In order to determine the phosphorus content of organic compounds, the solution being tested must first be digested to destroy the organic matter. In these soil extracts the amount of inorganic orthophosphate present was probably virtually identical with the total amount of inorganic phosphorus. Schmidt's review (86) suggests, however, that further investigation should be undertaken into the presence of pyro--and meta-phosphates.

The phosphorus determinations were made on appropriate aliquots. One aliquot was digested with two milliliters of 70 per cent $HClO_4$ to give total phosphorus present in the extract. Inorganic phosphorus was determined in an undigested aliquot of the fulvic acid fraction of the extract. After neutralization with 1:1 NH_4OH and 0.5N HCl using p-nitrophenol as indicator, phosphorus was determined by the method of Dickman and Bray (32) using concentrated $SnCl_2$ as a reducing agent as suggested by Mehta _et al_. (67). A technique proposed by Dyer and Wrenshall (34) and applied by Mehta _et al_. (67) permitted the measurements of inorganic phosphorus in coloured extracts. The spectrophotometer was set at a wavelength of 660 mu.

IV. RESULTS AND DISCUSSION

(A) Determination of Total Organic Phosphorus and Its Relation to Other Soil Properties

(a) Introduction

The methods of Mehta *et al*. and Kaila-Virtanen have given variable results (54, 106) as to which method would yield more organic phosphorus. VanDiest and Black (106) suggested that analyses by both methods on more soils were needed before making reliable inferences about the general superiority of one method or the other. The first objective of this investigation was, therefore, to apply these two methods on some Alberta soils.

Having thus obtained total organic phosphorus data it was thought of academic interest to expand this particular part of the investigation into an attempt to elucidate the place of organic phosphorus in the six Alberta soils under study. It is difficult to conclude from the literature if organic phosphorus is a variable or a characteristic fraction of soil organic matter. Many factors affect the phosphorus content of soil organic matter (11).

All the data collected for the twelve soil profiles are assembled in Tables 3a and 3b. A total of 56 samples was involved. Many difficulties were encountered while obtaining the various values. This was particularly true for the samples from the Ck and C horizons containing excess $CaCO_3$ and/or $CaSO_4$. The horizon designations are according to the latest Canadian system of classification (79). The

TABLE 3a VARIOUS SOIL PROPERTIES AND RATIOS OF SEVERAL ALBERTA SOIL SERIES

Soil Series	Horizon	Depth	pH	Org.C	Org.N	Non-sulphate S	Total P	Org.P		Org.P in Tot.P	C:Org.P
		in.		%	%	p.p.m.	p.p.m.	A* p.p.m.	B* p.p.m.	%	
1a. Brown	Ah	0- 4	6.8	3.6	0.23	233	472	153	141	29.9	255
(Shallow	Bm	5-12	6.9	1.2	0.11	209	445	153	135	30.3	89
Chin loam)	C	18-32	8.0	0.75	0.076	147	486	96	161	33.1	47
2a. Dark Brown	Ah	0- 8	6.8	4.5	0.28	301	610	178	196	32.1	230
(Lethbridge	Bm	8-18	7.2	1.3	0.11	190	499	165	164	32.9	79
sandy loam)	C	30	7.8	0.97	0.10	178	692	187	237	34.2	41
3a. Thin Black	Ah	0- 8	6.5	7.2	0.54	571	1109	615	548	49.4	131
(Airdrie	Bm	8-20	6.4	0.72	0.066	104	394	56	76	19.3	95
loam)	Ck	20-28	8.3	0.45	0.048	131	602	22	34	5.6	132
	C	34	8.0	0.20	0.024	103	631	49	43	6.8	47
4a. Black	Ah	0-11	6.6	8.9	0.77	875	1253	738	674	53.8	132
(Antler	Bm	11-34	6.6	1.4	0.13	213	473	195	170	35.9	82
loam)	Ck	34-46	8.5	0.88	0.093	281	661	171	100	15.1	88
	C	50	8.6	0.24	0.027	156	705	80	34	4.8	71
5a. Dark Grey	L-H	0- 2	6.9	24	1.5	961	1054	542	459	43.5	529
(Falun loam)	Ahe	2-12	6.3	3.6	0.27	161	550	235	159	28.9	226
	Btj	12-30	5.6	0.37	0.038	60	198	46	29	14.6	128
	Ck	36-40	7.4	0.27	0.028	89	421	44	35	8.3	77
	C	72	7.5	0.20	0.025	79	377	26	30	8.0	67
6a. Grey Wooded	L-H	-1- 0	6.7	21	0.99	672	988	392	366	37.0	574
(Cooking Lake	Ae	2-10	6.9	0.40	0.039	44	198	50	75	37.9	53
loam)	Bt	14-20	4.7	0.49	0.045	79	252	54	70	27.8	70
	Ck	40	7.9	0.30	0.032	109	507	39	61	12.0	49
	C	72	8.0	0.17	0.022	64	562	46	56	10.0	30

* A - Method by Mehta *et al*.
B - Method by Kaila-Virtanen

VERAL ALBERTA SOIL SERIES

Org.P		Org.P in Tot.P	C:Org.P	N:Org.P	S:Org.P	C : N : S : Org.P
A* p.p.m.	B* p.p.m.	%				
153	141	29.9	255	16	1.7	157 : 10 : 1.0 : 0.6
153	135	30.3	89	8	1.5	109 : 10 : 1.9 : 1.2
96	161	33.1	47	5	0.9	99 : 10 : 1.9 : 2.1
178	196	32.1	230	14	1.5	161 : 10 : 1.1 : 0.7
165	164	32.9	79	7	1.2	118 : 10 : 1.7 : 1.5
187	237	34.2	41	4	0.8	97 : 10 : 1.8 : 2.4
615	548	49.4	131	10	1.0	133 : 10 : 1.1 : 1.0
56	76	19.3	95	9	1.4	109 : 10 : 1.6 : 1.2
22	34	5.6	132	14	3.9	94 : 10 : 2.7 : 0.7
49	43	6.8	47	6	2.4	83 : 10 : 4.3 : 1.8
738	674	53.8	132	11	1.3	116 : 10 : 1.1 : 0.9
195	170	35.9	82	8	1.3	108 : 10 : 1.6 : 1.3
171	100	15.1	88	9	2.8	95 : 10 : 3.0 : 1.1
80	34	4.8	71	8	4.6	89 : 10 : 5.8 : 1.3
542	459	43.5	529	33	2.1	162 : 10 : 0.6 : 0.3
235	159	28.9	226	17	1.0	133 : 10 : 0.6 : 0.6
46	29	14.6	128	13	2.1	97 : 10 : 1.6 : 0.8
44	35	8.3	77	8	2.5	96 : 10 : 3.2 : 1.3
26	30	8.0	67	8	2.6	80 : 10 : 3.2 : 1.2
392	366	37.0	574	27	1.8	212 : 10 : 0.7 : 0.4
50	75	37.9	53	5	0.6	103 : 10 : 1.1 : 1.9
54	70	27.8	70	6	1.1	109 : 10 : 1.8 : 1.6
39	61	12.0	49	5	1.8	94 : 10 : 3.4 : 1.9
46	56	10.0	30	4	1.1	77 : 10 : 2.9 : 2.5

TABLE 3b VARIOUS SOIL PROPERTIES AND RATIOS OF SEVERAL ALBERTA SOIL SERIES

Soil Series	Horizon	Depth	pH	Org.C	Org.N	Non-sulphate S	Total P	Org.P		Org.P in Tot.P	C
		in.		%	%	p.p.m.	p.p.m.	A* p.p.m.	B* p.p.m.	%	
1a. Brown (Shallow Chin loam)	Ah	0- 5	6.8	2.9	0.20	258	541	208	186	34.4	
	Bm	5-12	7.3	1.4	0.12	162	505	194	189	37.4	
	C	12-24	8.0	0.94	0.090	156	679	121	117	17.2	
	Ck	24-36	8.1	0.51	0.051	144	523	83	79	15.1	
	IIC	36-48	8.0	0.26	0.029	130	550	93	75	13.6	
2a. Dark Brown (Lethbridge sandy loam)	Ah_1	0- 3	6.9	4.0	0.27	446	772	296	316	40.9	
	Ah_2	3-12	6.5	1.5	0.12	274	665	245	227	34.1	
	Bm	12-20	7.9	1.2	0.12	187	735	172	182	24.8	
	Ck	20-28	8.0	1.0	0.10	169	556	118	119	21.4	
	C	30	8.3	0.26	0.031	125	554	79	92	16.6	
3a. Thin Black (Airdrie loam)	Ah	0- 5	6.6	4.5	0.33	489	910	417	334	36.7	
	Bm	5-16	6.8	1.3	0.12	259	420	190	167	39.8	
	Ck	16-24	8.0	1.1	0.11	234	671	181	144	21.5	
	C	24-36	8.2	0.26	0.028	157	631	43	52	8.2	
4a. Black (Antler loam)	Ah	0-13	6.0	6.9	0.48	519	1180	548	540	45.8	
	AB	13-22	5.8	0.69	0.057	125	379	101	131	34.6	
	Bm	22-36	6.0	0.56	0.051	110	409	94	112	27.4	
	Ck	36-50	8.1	0.42	0.040	103	574	92	82	14.3	
	C	56-60	8.2	0.10	0.011	63	559	18	67	12.0	
5a. Dark Grey (Falun loam)	L-H	-2- 6	6.5	19.5	1.4	651	942	328	329	34.9	
	Ahe	6-10	6.3	2.1	0.17	119	559	167	161	28.8	
	Bt̄j	24	5.8	0.37	0.035	91	424	117	114	26.9	
	BC	40	5.6	0.17	0.018	81	426	56	59	13.8	
	C_1	48	6.8	0.15	0.016	69	534	58	65	12.2	
	C_2	60-72	7.5	0.095	0.011	63	534	39	76	14.2	
6a. Grey Wooded (Cooking Lake loam)	L	-3--1	6.4	36	1.9	1339	1710	649	638	37.3	
	FH	-1- 0	5.2	22	1.2	708	1225	503	452	36.9	
	Ae	0- 5	6.1	0.47	0.044	72	200	118	110	55.0	
	Bt_1	5-11	4.8	0.69	0.066	109	505	127	105	20.8	
	Bt_2	11-24	4.2	0.31	0.032	63	271	69	76	28.0	
	Bm	24-29	7.1	0.25	0.027	56	486	103	78	16.0	
	C	50	7.5	0.18	0.021	41	474	31	66	13.9	

* A - Method by Mehta et al.
B - Method by Kaila-Virtanen

SEVERAL ALBERTA SOIL SERIES

Org.P		Org.P in Tot.P	C:Org.P	N:Org.P	S:Org.P	C : N : S : Org. P
A* p.p.m.	B* p.p.m.	%				
208	186	34.4	156	11	1.4	145 : 10 : 1.3 : 0.9
194	189	37.4	74	6	0.9	117 : 10 : 1.4 : 1.6
121	117	17.2	80	8	1.3	104 : 10 : 1.7 : 1.3
83	79	15.1	65	6	1.8	100 : 10 : 2.8 : 1.5
93	75	13.6	35	4	1.7	90 : 10 : 4.5 : 2.6
296	316	40.9	127	9	1.4	148 : 10 : 1.7 : 1.2
245	227	34.1	66	5	1.2	125 : 10 : 2.3 : 1.9
172	182	24.8	66	7	1.0	100 : 10 : 1.6 : 1.5
118	119	21.4	84	8	1.4	100 : 10 : 1.7 : 1.2
79	92	16.6	28	3	1.4	84 : 10 : 4.0 : 3.0
417	334	36.7	135	10	1.5	136 : 10 : 1.5 : 1.0
190	167	39.8	78	7	1.6	108 : 10 : 2.2 : 1.4
181	144	21.5	76	8	1.6	100 : 10 : 2.1 : 1.3
43	52	8.2	50	5	3.0	93 : 10 : 5.6 : 1.9
548	540	45.8	128	9	1.0	144 : 10 : 1.1 : 1.1
101	131	34.6	53	4	1.0	121 : 10 : 2.2 : 2.3
94	112	27.4	50	5	1.0	110 : 10 : 2.2 : 2.2
92	82	14.3	51	5	1.3	105 : 10 : 2.6 : 2.1
18	67	12.0	15	2	0.9	91 : 10 : 5.7 : 6.1
328	329	34.9	593	43	2.0	139 : 10 : 0.5 : 0.2
167	161	28.8	130	11	0.7	124 : 10 : 0.7 : 0.9
117	114	26.9	32	3	0.8	106 : 10 : 2.6 : 3.3
56	59	13.8	29	3	1.4	94 : 10 : 4.5 : 3.3
58	65	12.2	23	2	1.1	94 : 10 : 4.3 : 4.1
39	76	14.2	13	1	0.8	86 : 10 : 5.7 : 6.9
649	638	37.3	564	30	2.1	189 : 10 : 0.7 : 0.3
503	452	36.9	509	27	1.6	183 : 10 : 0.6 : 0.4
118	110	55.0	43	4	0.7	107 : 10 : 1.6 : 2.5
127	105	20.8	66	6	1.0	105 : 10 : 1.7 : 1.6
69	76	28.0	41	4	0.8	97 : 10 : 2.0 : 2.4
103	78	16.0	32	3	0.7	93 : 10 : 2.1 : 2.9
31	66	13.9	27	3	0.6	86 : 10 : 2.0 : 3.1

data are organized in their present form for convenience only. Soil analyses were done on only two representative profiles from each soil series, although Jackson (51, p. 17) recommended that at least three profiles should be sampled and analyzed before making generalizations. If certain trends show any amount of persistency a more thorough study will be required before definite conclusions can be made.

(b) Total organic phosphorus

The above mentioned two acid-alkaline extraction methods of Mehta et al. and Kaila-Virtanen were used to determine the total organic phosphorus content. For several reasons, as will be shown later, the Kaila-Virtanen procedure was thought to be slightly the better method and was, therefore, used for the subsequent correlation and ratio calculations. The overall averages of the total organic phosphorus values were as follows:

Samples of	Mehta et al. (p.p.m.)	Kaila-Virtanen (p.p.m.)
Table 3a	181	169
Table 3b	177	173
Tables 3a + 3b	178	171
Tables 3a + 3b - pH < 7.0	248	234
pH > 7.0	92	95

Kaila and Virtanen (54) pointed out that none of the methods used up until 1955 gave results of high accuracy. They stated further that the amounts of organic phosphorus could not be reported at greater precision than 10-20 p.p.m. of organic phosphorus. This is important when comparing the results of any two methods to determine which method is superior. Accordingly, from the foregoing data it may be concluded that, for the soils under study, both methods were similar. However, this conclusion is in disagreement with both Kaila and Virtanen's (54) and VanDiest and Black's (106) findings. Actually, it is not merely a matter of analyzing more and more soils, but also of obtaining a better understanding of the reactions involved and the forms of organic phosphorus determined. As Anderson (6) pointed out, there may be distinct qualitative differences in the phosphate esters present in various soil types or soils of different regions. This indicates that completely different approaches are needed, such as differential thermal--, infrared--, electrophoretic--, and chromatographic analyses. After the types of compounds have been identified, specific quantitative methods can be developed. No one method can possibly estimate all the phosphate esters present in the various horizons of soils, particularly when no method has yet been developed which will extract all the organic matter present in a soil.

It should be noted, that except for the Brown and Dark Brown soils, the amount of organic phosphorus found in the upper horizons appears to be consistently greater by the Mehta et al. method than by the Kaila-Virtanen procedure. For the lower horizons, the two methods are either equal, or the Kaila-Virtanen procedure generally gives slightly higher values. These lower horizons all have a pH of 7.0 or greater, yet the foregoing overall averages of the total organic phosphorus values do not show any appreciable differences between the two methods.

As reported in the literature review, one of the reasons for pre-treating the soil with an acid solution before alkaline extraction was for the removal of $CaCO_3$ and exchangeable calcium, which would render part of the organic phosphorus insoluble during the alkaline extraction. Total organic phosphorus values obtained by the Mehta et al. and Kaila-Virtanen methods for the Ck of a Cooking Lake loam are 39 and 61 p.p.m. respectively, and for the Cks of a Black Solonetz 6 and 53 p.p.m. respectively. When 500 ml. of a 1N NH_4OAc solution of pH 7.0 were leached through 25 gm. of the two soils at a rate of one drop every three seconds, the total organic phosphorus values of the Ck of the Cooking Lake loam and Cks of the Black Solonetz by the Mehta et al. method became 76 and 66 p.p.m. respectively. No change occurred with the Kaila-Virtanen

procedure. However, these differences between the two methods were not consistent, otherwise the overall difference between the two methods at pH 7.0 would be at least 20 p.p.m., which was not the case. It was noted in some cases that the Mehta et al. method did not completely remove the $CaCO_3$ and exchangeable calcium. The summary of steps given in Table 2 may, at least partially, explain these results. The acid extraction of the Kaila-Virtanen procedure was filtered off, while that of the Mehta et al. method was only centrifuged off. As will be reasoned later, filtration seems to be a more efficient method for removing the calcium than centrifugation. The acid and alkaline extracts of the Mehta et al. method were tested separately for organic phosphorus content to see if possible precipitation of organic phosphorus by calcium took place. This did not lead, however, to an increase in the total organic phosphorus values of the soils analyzed.

It may be postulated that fewer forms of organic phosphorus are present in the Brown and Dark Brown surface horizons than in the Thin Black, Black, Dark Grey, and Grey Wooded soils. Prolonged periods of drought particularly in the Brown and Dark Brown soil zones tend to retard the rate of decomposition of organic matter. Joffe (53, p. 275) pointed out, that the intermittent wetting and drying effects, due to erratic summer rainfall, accentuate the aging, stabilizing, and gelling of the organic colloids. These substances then remain highly resistant to decomposition. As a result less diversification takes place

into different forms of phosphate esters by decomposing and synthesizing activities of microorganisms. It is probable that any extraction procedure will separate out several forms of organic phosphorus. For example, in the case where two different methods are used, certain forms of organic phosphorus will be extracted by both procedures, while other forms will be obtained by one method and not by the other. Therefore, when little or no differentiation took place, little or no differences between the two methods should occur, at least in the upper horizons. The present data seem to support this contention.

The consistent difference between the two methods for the averages of the upper horizons of the Thin Black, Black, Dark Grey, and Grey Wooded soils may have been the result of a variety of forms of organic phosphorus present as explained in the previous paragraph. It may also have been the result of occlusion by adsorption of inorganic phosphorus with the "humic acid". In the Mehta et al. method the combined acid and alkaline extracts are shaken and a sample is digested prior to determining total phosphorus extracted. After settling of the "humic acid" (soluble in alkali, but insoluble in acid) a sample of the supernatant liquid or "fulvic acid" (alkali-soluble and acid-soluble) is taken and used for the determination of inorganic phosphorus extracted. The difference between the two values is the organic phosphorus extracted from the soil. If a certain

amount of inorganic phosphorus is adsorbed by the "humic acid", the values for total organic phosphorus will be too high.

In an attempt to prove this hypothesis the following equations were formulated:

Total P in fulvic acid-Inorganic P in fulvic acid = Organic P in fulvic acid.

Total P in whole extract-Inorganic P in fulvic acid = Organic P in whole extract.

Total P in humic acid-(Organic P in whole extract-Organic P in fulvic acid) = Inorganic P in humic acid.

This approach required total phosphorus determinations of the "fulvic -" and "humic acid" fractions. Seven surface horizon samples were selected to supply data for above equations, because these horizons released considerable amounts of "humic acid". The results in quadruplet were very inconclusive, but this is not surprising because each value contains an absolute error of at least ±20 p.p.m. Consequently, the inorganic phosphorus in the humic acid fraction will have, as a result of four subtractions, an absolute error of ±80 p.p.m.

The total organic phosphorus, by both methods, decreased with depth as is illustrated in Figure 2. The plotted values are the means of the two methods as reported in Tables 3a and 3b. The soil series are again kept separate for clarity. For all the profiles sampled, the total organic phosphorus was less than 200 p.p.m. at depths

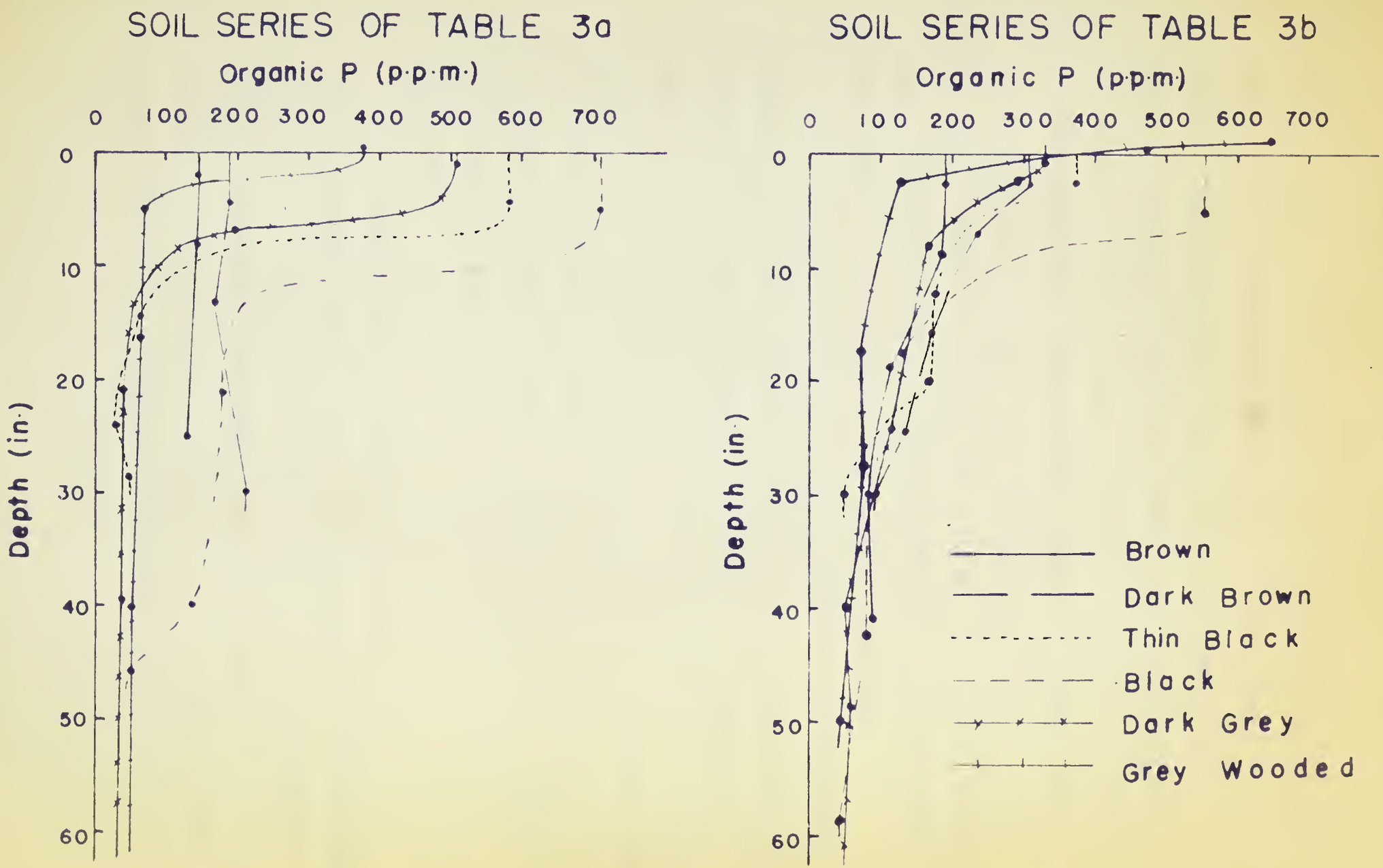

Figure 2. Schematic distribution of total organic phosphorus in the various soil profiles sampled.

greater than one foot. The only exception was the Dark Brown soil of Table 3a with 212 p.p.m. at a depth of 30 inches. The relation of the six soil series to each other is generally the same in both diagrams of Figure 2. The decrease of the organic phosphorus content of the Brown and Dark Brown soil series with increased depth is of course much more gradual than for the Thin Black, Black, Dark Grey, and Grey Wooded soil series. The chief reason being that the Brown and Dark Brown soils did not contain much organic phosphorus in the surface horizons.

The organic phosphorus content in the upper horizon increased from the Brown to the Black, and decreased again with the Dark Grey (Figure 2). This was true for both methods. The averages of the four values for each soil are 172, 247, 479, 625, and 415 p.p.m. for the Brown, Dark Brown, Thin Black, Black, and Dark Grey soils, respectively. In terms of per cent organic phosphorus of total phosphorus these figures were 32, 37, 43, 50, and 39 per cent, respectively.

The organic matter content increases from Brown to Black soils with the Dark Grey soils generally being lower in organic matter than the Black soils (17). The total organic phosphorus data shown in Tables 3a and 3b followed the same trend as organic matter content. The organic carbon and nitrogen in the Dark Grey soils,

however, show an increase over the Black soils, because the L-H horizons are actually organic horizons without any mineral matter. An increase in energy-yielding material will give an increase in microorganisms thereby increasing the amount of organic phosphorus. In the L-H horizons of the Dark Grey soils fungi start to appear which probably are the cause of less organic phosphorus formation.

In order to determine non-sulphate sulphur the soil samples were digested and washed with hot NH_4Cl, and washed twice with a 100 ml. portion of hot, distilled water. Samples of the Ck and C horizons, particularly of the Fálun and Cooking Lake loams, released an unexpected large amount of humic material with the first 100 ml. portion of hot, distilled water. Upon dializing, evaporating to dryness, and wet ashing, the presence of organic phosphorus was demonstrated. This, together with the fact that up to 100 p.p.m. of organic phosphorus was found in the C horizon allowed several suggestions to be made with regard to this organic phosphorus:

1. Remains of roots and microorganisms, and agitating activities of the soil fauna gradually moving the phosphorus down the profile, are likely reasons for the high organic phosphorus contents at these depths.

2. The parent material may have come from developed soils. The glaciers, which at one time covered most

of Alberta, carried and deposited a highly variable mixture of previously formed soils and freshly reduced rock material, resulting in a certain amount of organic matter being present in the parent material of the present soils.

3. The finely ground rock material in this till may have occluded organic compounds during formation in early geologic times (100).

4. The horizons of these profiles which were sampled as C horizons may still have been the B horizons as far as organic phosphorus was concerned.

(c) Total phosphorus

The total phosphorus in the Ah horizon of the Brown and Dark Brown soil series is not much different from that present in the C horizon. The high total phosphorus in the surface horizons of the other soils correlates with the high amounts of the organic phosphorus present. At lower depths the total phosphorus is fairly constant down the profile for all soils.

(d) Ratios and relationships

The relationships of total organic phosphorus with total phosphorus, organic carbon, organic nitrogen, and non-sulphate sulphur are shown in Table 4, which tabulates the different correlation coefficients (r) and coefficients of determination (r^2 x 100). Three values are given in each comparison. The first one involves all values, while

TABLE 4. RELATIONSHIP OF TOTAL ORGANIC PHOSPHORUS WITH TOTAL PHOSPHORUS, ORGANIC CARBON AND NITROGEN; AND NON-SULPHATE SULPHUR

Comparison	r	r^2 x 100
Organic P vs. Total P	0.875	76.6
below pH 7.0	0.952	90.6
above pH 7.0	0.302	9.1
Organic P vs. Organic C	0.768	59.0
below pH 7.0	0.742	55.1
above pH 7.0	0.842	70.9
Organic P vs. Organic N	0.810	65.6
below pH 7.0	0.786	61.8
above pH 7.0	0.844	71.2
Organic P vs. Non-sulphate S	0.915	83.7
below pH 7.0	0.920	84.6
below 500 p.p.m. S	0.949	90.1
above 500 p.p.m. S	0.463	21.4
above pH 7.0	0.543	29.5
Org.C/Org.N vs. Org.C/Org.P	0.840	70.6
below pH 7.0	0.839	70.4
above pH 7.0	0.451	20.3
Org.C/Org.S vs. Org.C/Org.P	0.938	88.0
below pH 7.0	0.948	89.9
above pH 7.0	0.365	13.3

the second and third involve the values either below or above pH 7.0. The values for non-sulphate sulphur at pH less than 7.0 are divided again into below and above 500 p.p.m. of non-sulphate sulphur. The data depicted in Figure 2a clarify the reason for the use of this value of 500 p.p.m. non-sulphate sulphur. A more detailed discussion of this Figure will be given on page 70.

The square of a correlation coefficient gives a measure of the extent to which the two variates involved are associated. Or, as Finney (39) points out, it is essentially the proportion of the variability in one variate that can be accounted for by the variations in the other. For example, the correlation coefficient of organic versus total phosphorus is 0.875. This means in terms of $r^2 \times 100$, that 76.6 per cent of the variation in organic phosphorus can be regarded as associated with variation in total phosphorus, whereas 23.4 per cent is residual variation independent of total phosphorus. Finney (39) points out further that coefficients greater than 0.85 are seldom found, since this means that 70 to 75 per cent or more of the variations in the two measurements are then associated. This will only be true for pairs of measurements having some obviously important physical connection. The words "close correlation" should not be used in relation to any correlation coefficient smaller than 0.700 which represents about 50 per cent dependence of variation.

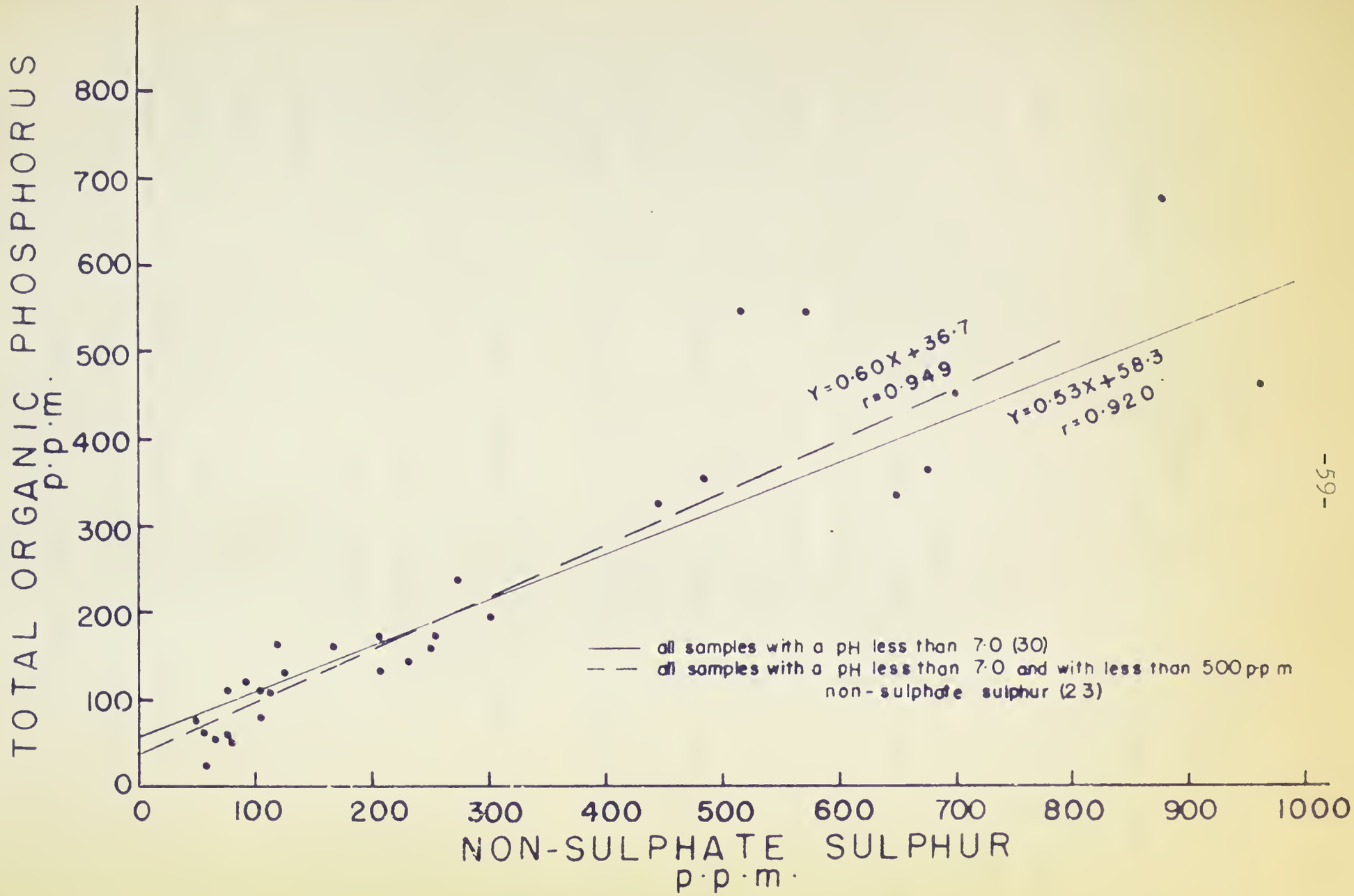

Figure 2a. The relationship between total organic phosphorus and non-sulphate sulphur for samples with pH less than 7.0.

Walker (110) contended that the phosphorus in the soil organic matter had to come from the parent material. A high correlation between organic and total phosphorus was therefore not unexpected (Figure 3), especially in the upper horizons which are somewhat acidic. These horizons are acidic mainly as a result of the presence of organic matter. Various microbes and fungi, living in and utilizing organic matter, produce organic acids. Further, as a result of the decomposition of plant and animal residues, and soil humus, an increase in the evolution of carbon dioxide is obtained, thereby increasing the carbon dioxide content of the soil atmosphere. Both, organic acids and carbon dioxide content of the soil atmosphere, increase the hydrogen-ion concentration of soil that contains organic matter.

There was a weak tendency for the organic phosphorus as percentage of total phosphorus to decrease as the pH increased. This relationship, however, was not statistically significant. A similar weak tendency was illustrated by Thompson (102, p. 251) for virgin and cultivated surface samples. These data are, therefore, not completely comparable with those in the present study. On the other hand, the correlation between organic phosphorus versus total phosphorus was strongly affected by pH (Table 4).

As would be expected, a close association existed between organic carbon and organic nitrogen, non-sulphate sulphur and organic nitrogen, and non-sulphate sulphur and organic carbon

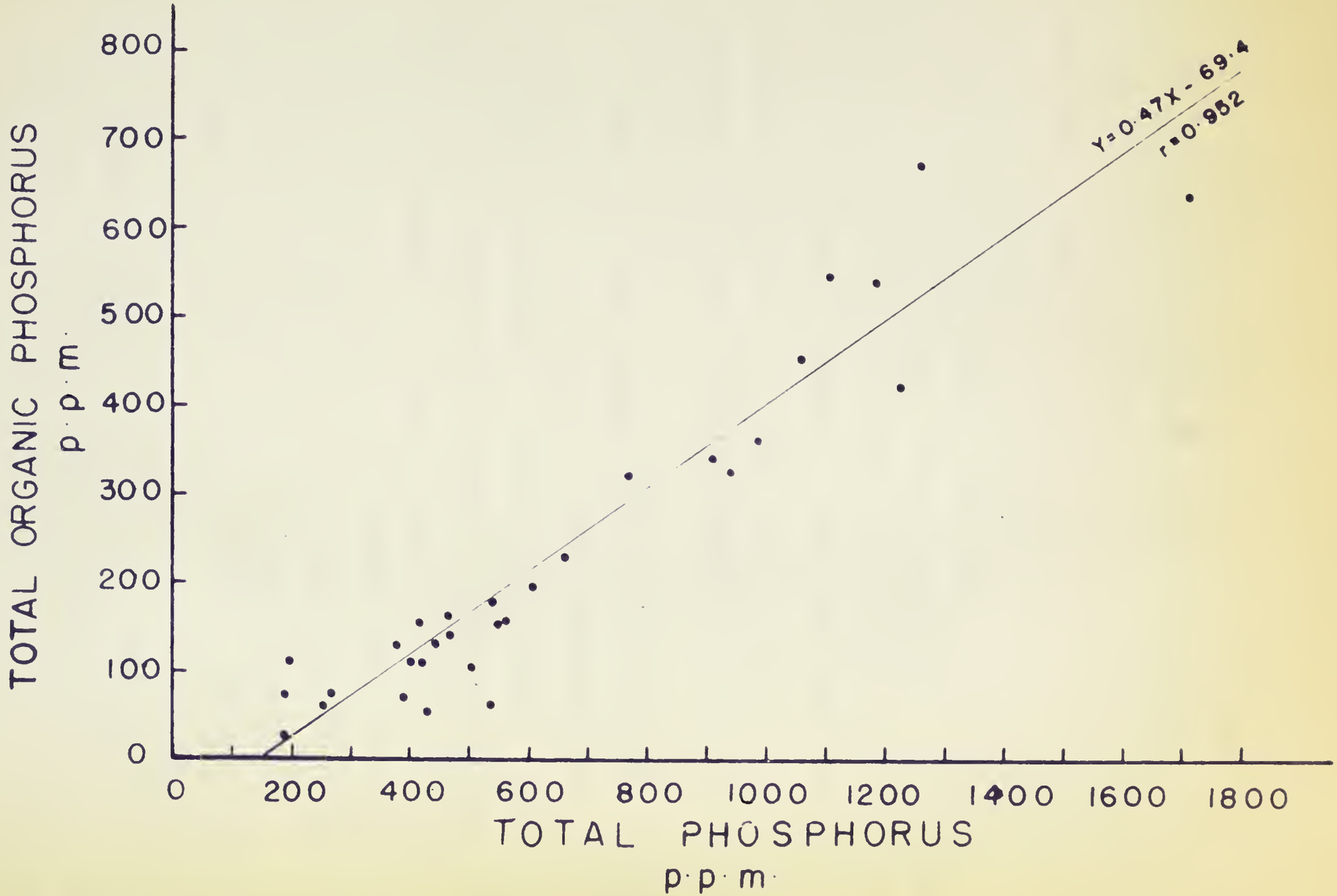

Figure 3. The relationship between total organic phosphorus and total phosphorus for samples with pH less than 7.0.

for samples from all soil series (Figures 4, 5, and 6). The values from the L-H horizons are not included in these Figures, because the absence of mineral matter gives these horizons a completely different composition as compared with the other horizons. The C/N ratios for all the other horizons vary from slightly less than 10 in the lower to 16 in the upper horizons. Non-sulphate sulphur particularly is highly correlated with both organic carbon and nitrogen, which is reasonable because all three are considered to be essential constituents of soil organic matter. This indicates that most of the non-sulphate sulphur is present in organic forms.

The association between organic phosphorus and organic carbon and nitrogen should be better, theoretically, than is indicated in Table 4. Soils high in organic carbon and nitrogen should also be high in organic phosphorus. The correlations, as shown in Figures 7 and 8, where the values for the L-H layers are omitted, are particularly high for the samples with a pH of less than 7.0. These correlations are much higher than those reported by Williams _et al_. (119), but are similar to those of Walker and Adams (112). The latter reported high correlations for organic P/organic N ratios at all three layers 0-7 inch, 7-14 inch, and 14-21 inch depths. From both Williams' (116) and Williams and Steinbergs' (118) data, it is evident that in general the organic phosphorus increased with increasing

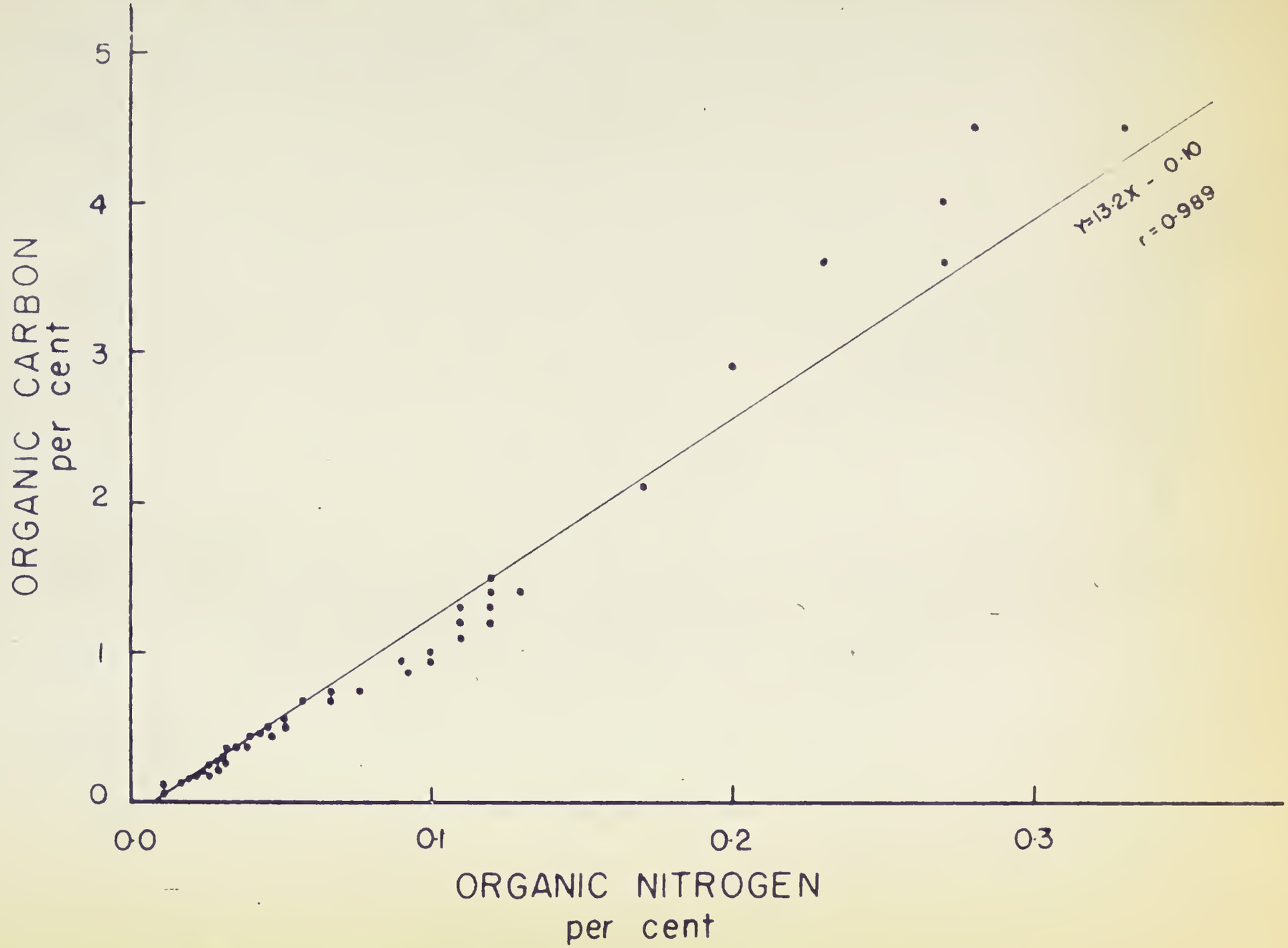

Figure 4. The relationship between organic carbon and organic nitrogen.

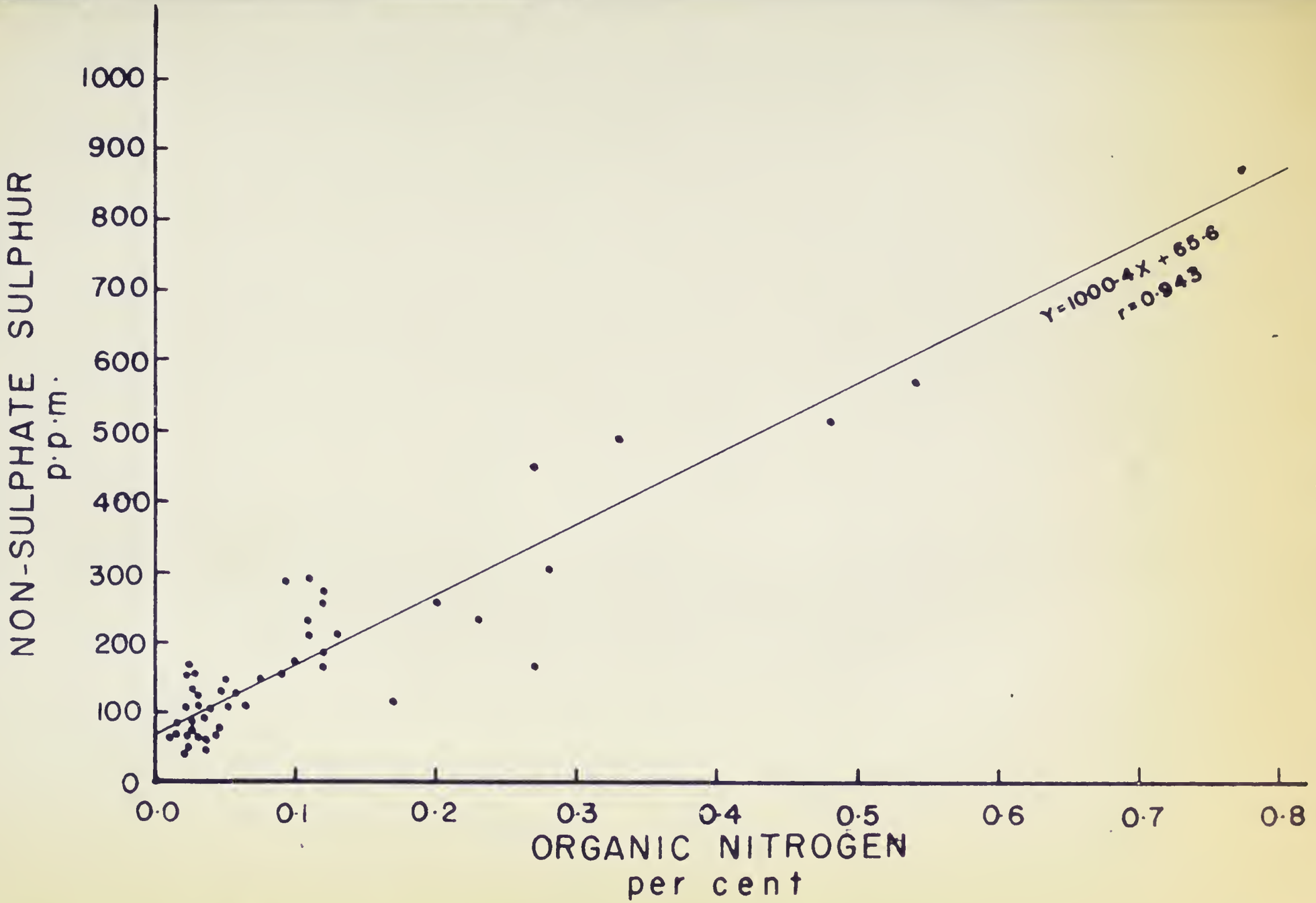

Figure 5. The relationship between non-sulphate sulphur and organic nitrogen.

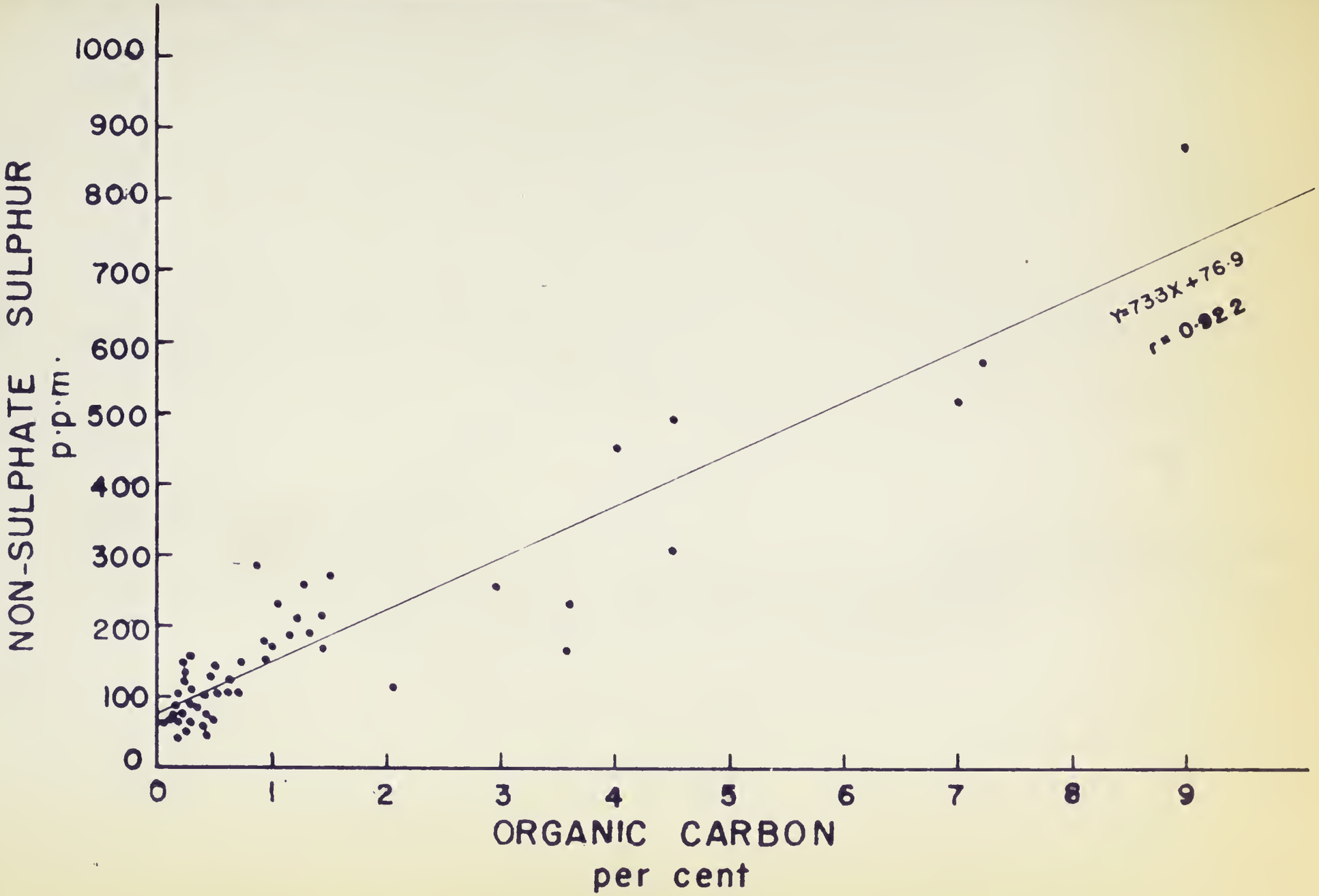

Figure 6. The relationship between non-sulphate sulphur and organic carbon.

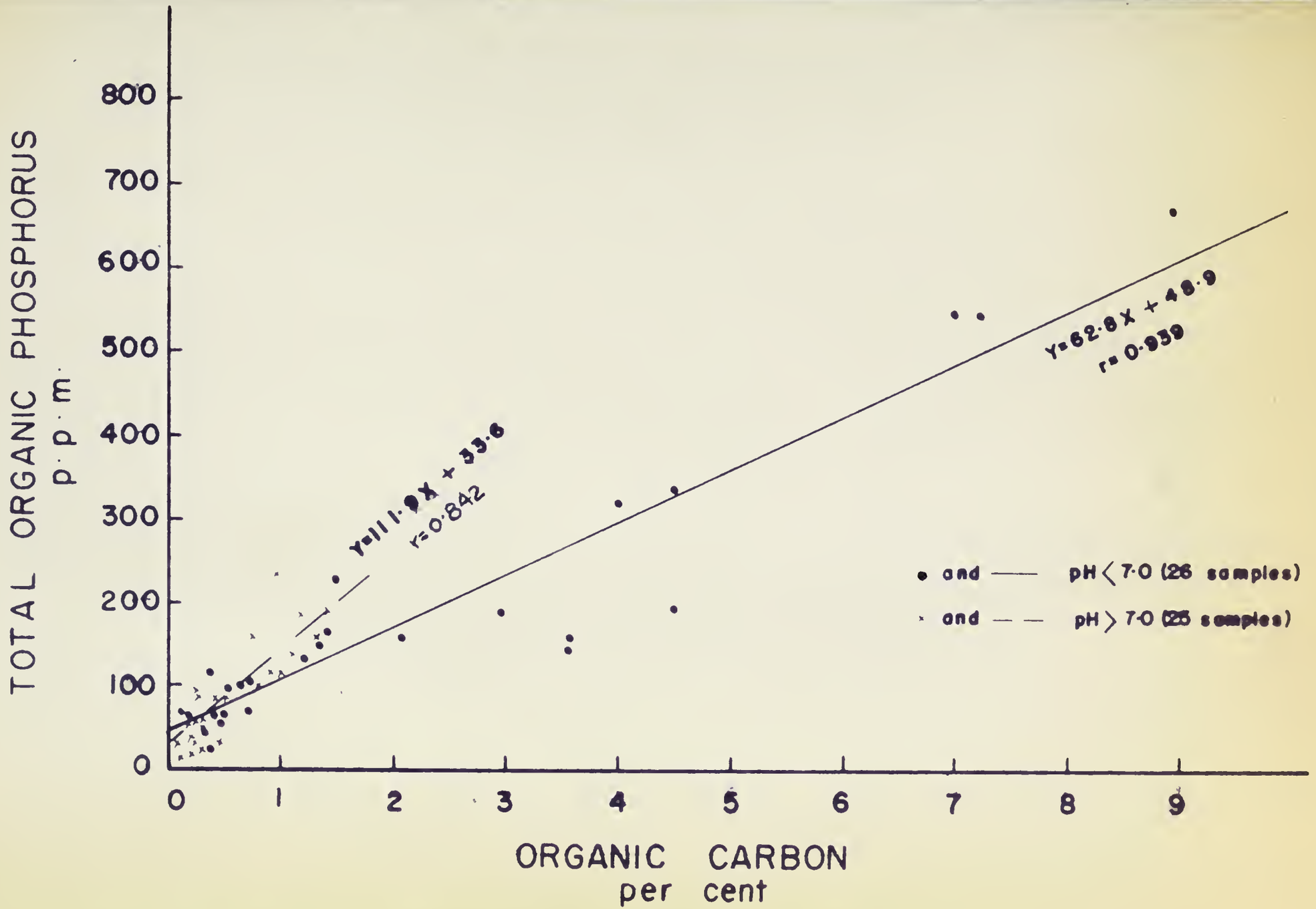

Figure 7. The relationship between total organic phosphorus and organic carbon.

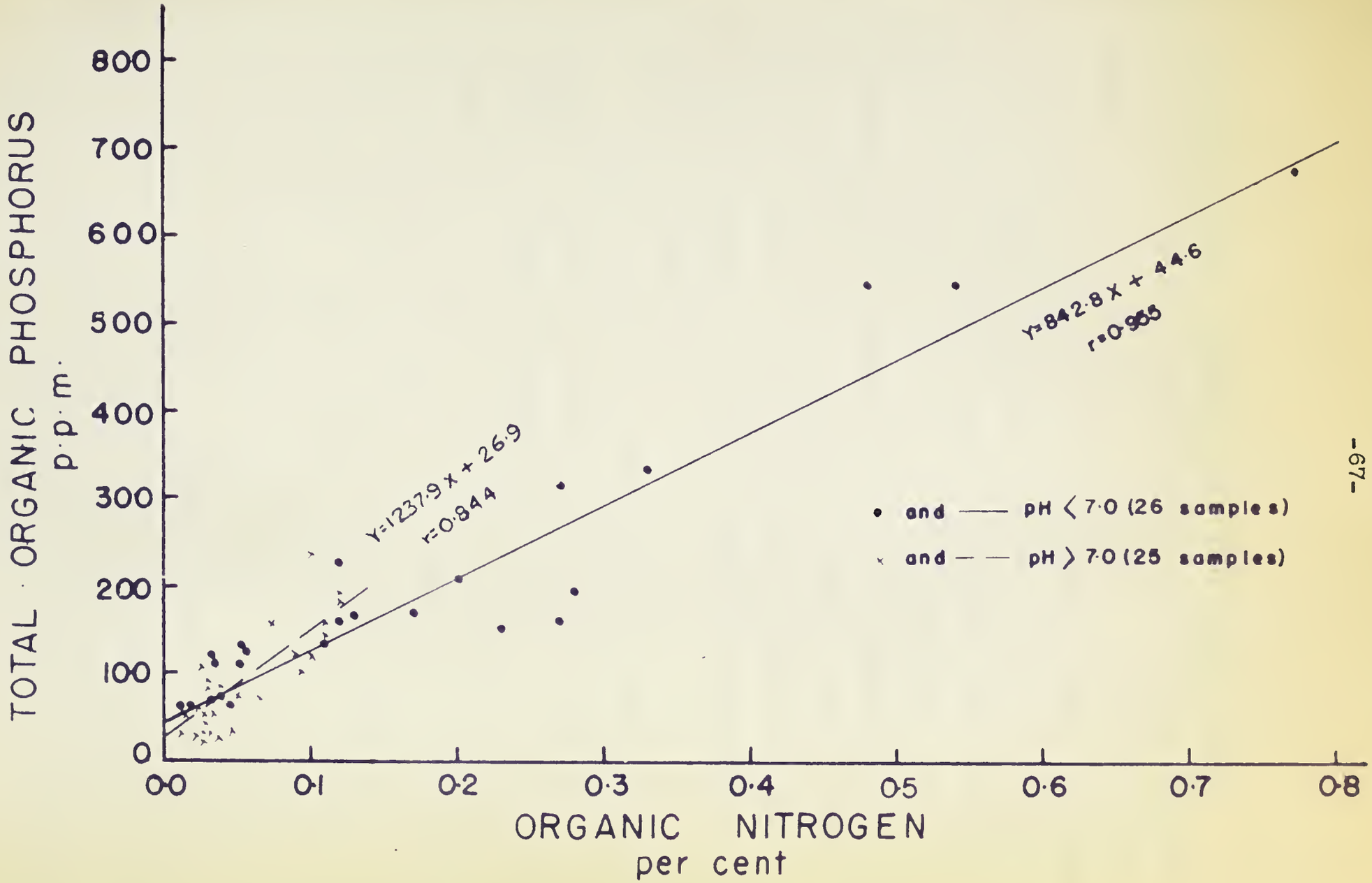

Figure 8. The relationship between total organic phosphorus and organic nitrogen.

nitrogen content. However, the relationship between nitrogen and organic phosphorus was not as close as that between nitrogen, carbon, and sulphur. It seems a matter of interest, in spite of the tremendously high correlations, that the values of Figures 4, 5, and 6 show a more even distribution along the lines of regression than those of Figures 7 and 8. Dean (31) on the other hand, after attempting a fractionation of the soil phosphorus and finding that organic phosphorus increased significantly with the carbon contents of the soil, implied that the organic phosphorus is mainly a function of plant and biological activities.

Thompson et al. (104) reported high correlations and positive associations between the ratios of nitrogen and carbon to organic phosphorus with soil pH. No such high correlations, however, were found in the present study. The representative samples of the Brown, Dark Brown, Thin Black, and Black soil series do show some degree of relationship. The correlation coefficients are -0.446 and -0.276 for the organic C/organic P : pH and organic N/organic P : pH ratios, respectively. The correlation coefficient of -0.446 is significant at the one per cent level (97, p. 174), because it is based on 33 observations. But, in view of the previous discussion on correlation data, these values are far from acceptable. A correlation

coefficient of -0.446 means that only 20 per cent of the variation in pH is accounted for by variation in the organic C/organic P ratios. Finney (39) states aptly that such correlation coefficients "scarcely indicate a degree of correlation close enough to be much use in prediction". The difference between the present and Thompson et al. associations (104) is most likely due to different types of soil samples used. The latter used surface soils only, that contained organic matter over the whole pH range represented. In the study under consideration, samples from all horizons were utilized and the higher pH values came from samples containing very little organic matter.

Most accounts in the literature deal with soils from cultivated land. Thompson et al. (104) showed that the rates of mineralization of organic carbon, nitrogen and phosphorus were greater in virgin soils than in cultivated soils. It was postulated that a fraction of each of these constituents in the virgin soils was relatively more susceptible to mineralization than was the remainder after cultivation. Some of the cultivated New Zealand, Australian and Scottish soils which are reported on in the literature (112, 113, 118, 119) may have contained a certain amount of easily mineralizable organic phosphorus. Cultivated soils could end up, under such conditions, with less organic phosphorus, proportionally, than would be the case when mineralization of organic nitrogen and carbon

is associated positively with organic phosphorus. This then would result in relationships of organic nitrogen and carbon with organic phosphorus which would not be as good in cultivated as in the comparable virgin soils.

Williams et al. (119) inferred that the relationships of organic carbon and nitrogen with organic phosphorus were not easy to understand. They did not regard phosphorus as an integral part of the bulk organic matter, partly as the result of varying and more independent accumulation of resistant organic phosphorus compounds such as inositol phosphates. Several other accounts (8, 60, 96, 101), on the other hand, showed that inositol phosphates comprised only a small proportion of the total organic phosphorus. Anderson (4) indicated that about 20-40 per cent of the organic phosphorus in acid soils may be present in this form, mainly the hexaphosphate form. Thus, the argument of the importance of inositol phosphate content in soils is still not settled.

It is apparent from Figure 9 that the relationship of organic phosphorus with non-sulphate sulphur is remarkably high, particularly in samples with a pH less than 7.0 and containing less than 500 p.p.m. of non-sulphate sulphur. It could be possible that all four elements, organic carbon, nitrogen and phosphorus; and non-sulphate sulphur, are combined in various aliphatic

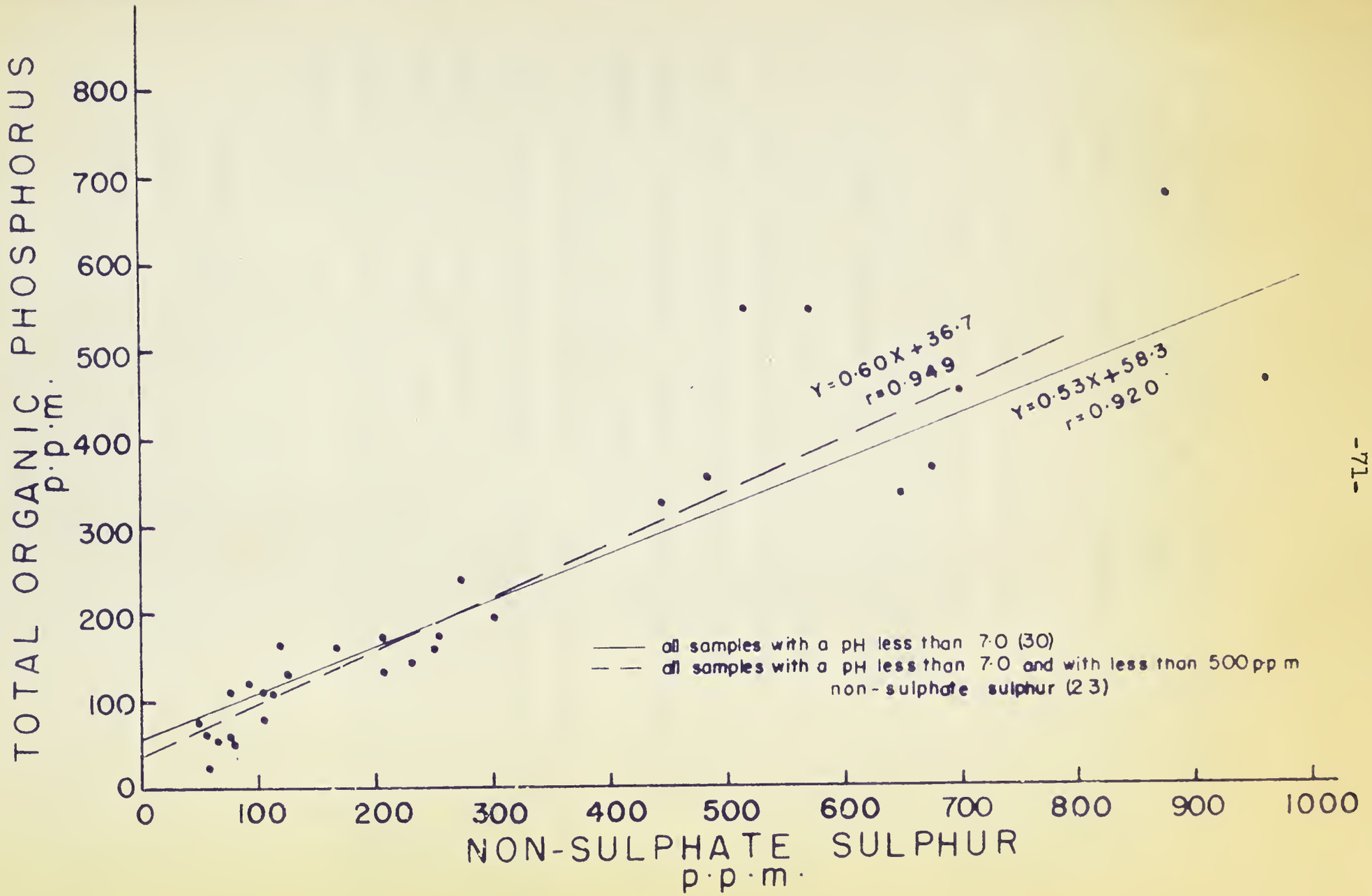

Figure 9. The relationship between total organic phosphorus and non-sulphate sulphur for samples with pH less than 7.0.

and/or aromatic compounds. This would particularly be conceivable if the soils under study did not contain appreciable amounts of inositol phosphate, and the work of Thomas and Lynch (101) seemed to imply this. The close relationships of organic phosphorus with organic nitrogen, organic carbon, and non-sulphate sulphur, all within the realm of organic matter, should substantiate such a hypothesis. Williams et al. (119) noted, on the other hand, that in the case of certain Scottish soils the ratios of organic phosphorus to organic matter were more variable than those of non-sulphate sulphur to organic matter. Another possibility, however, lies in the incomplete extractions of organic phosphorus.

The tabulation of Table 1, which lists some of the many organic carbon, organic nitrogen, organic phosphorus, and non-sulphate sulphur ratios cited in the literature, shows rather clearly that variations in these ratios do exist. The data of the present study permit the following tabulation:

	C : N : S	C : N : P
Overall mean	113 : 10 : 2.3	113 : 10 : 1.8
pH $<$ 7.0	129 : 10 : 1.6	129 : 10 : 1.4
pH $>$ 7.0	95 : 10 : 3.1	95 : 10 : 2.2

The ratios for the alkaline samples are not comparable to those reported in the literature for alkaline soils, because most of the ratios of Table 1 were for surface

horizons. The organic matter regime is rather different for alkaline surface samples than for alkaline samples taken at lower horizons. Both the C:N:S and C:N:P ratios for the samples with pH less than 7.0 fall well within the ranges reported in Table 1.

(e) Summary

The foregoing presentation seems to justify several conclusions. Two methods for determining total organic phosphorus, which according to the literature gave variable results, extracted similar amounts of organic phosphorus from six Alberta soils. The correlations as shown in the various Figures were all exceptionally high suggesting that organic phosphorus formed an integral part of organic matter.

It was postulated that fewer forms of organic phosphorus were present in the Brown and Dark Brown surface horizons than in the Thin Black, Black, Dark Grey, and Grey Wooded soils. High calcium content and impeded microbiological activities retard the rate of decomposition of organic matter in the Brown and Dark Brown surface horizons resulting in less diversification into different forms of phosphate esters. The need for extensive and accurate knowledge of the different forms of organic phosphorus present in the different horizons of any soil has become most pressing indeed. Although interesting data have been collected on Alberta soils, studies of this nature do not

furnish information regarding forms of organic phosphorus in soil. New approaches, as has been suggested, must be undertaken. It is no longer a question of a few, simple, rapid extractions at relatively low cost, but painstaking, expensive, time consuming, step by step analyses will have to be carried out.

(B) Comparison and evaluation of methods for determining total organic phosphorus

The previous section of this investigation evaluated two extraction methods which had been disputed in the literature as to which one would yield the greater amount of organic phosphorus. However, several other methods have been used by various workers for determining the total organic phosphorus content of soils. In the second part of this study five methods, the two previously mentioned extraction procedures, an additional extraction procedure (49), and two ignition procedures (59, 84), were selected for further study.

Several approaches were taken. First, it was thought desirable to gain some general knowledge about the five methods selected by merely applying them to a series of soil samples and analyzing the results statistically. Secondly, the effectiveness with which these procedures recovered two forms of organic phosphorus added to the soil was investigated. Finally, since extraction methods

for determining soil organic phosphorus are rather laborious, attention was focused on the more rapid and simple dry combustion procedures.

(a) Comparison of methods

A comparison of the different steps within each method has been presented in Table 2. The methods by Legg-Black and Saunders-Williams ignite the soil, thereby changing the organic phosphorus to the inorganic form. Legg-Black's method employs an ignition temperature of 240°C followed by strong acid extraction, whereas the Saunders-Williams' method uses 550°C followed by a mild acid extraction.

Two of the other procedures, Hayashi-Takijima and Kaila-Virtanen, are relatively mild extraction procedures. This is in contrast with the Mehta et al. method, which uses concentrated HCl as an acid pretreatment. Another contrast is the way the acid extraction is drawn off in that the Hayashi-Takijima and Kaila-Virtanen procedures employ filtration and the Mehta et al. method centrifugation. Theoretically filtration should be better. For reversible reactions a homogeneous, dynamic equilibrium can be illustrated by the following equation:

$$A + B \rightleftharpoons C + D$$

In order to force the reaction to the right either products C and/or D should be removed by techniques such as filtration or centrifugation. As was stated in the literature

review, one of the reasons for the acid pretreatment was to remove $CaCO_3$ and exchangeable calcium. Filtration and washing are better suited to the removal of reaction products.

Ovendry samples of five horizons, namely L-H, Ah, Bt_1, Bt_2, and C of a Black Solodized Solonetz, were subjected in duplicate to the various manipulations of the three acid-alkaline extraction and two ignition type methods. Table 5 summarizes the results of total organic phosphorus, while a summary of the Analysis of Variance is given in Table 6.

The source of Variation for Treatment has been broken down into Methods and Soil. Soil, of course, accounts for a great percentage of the variation due to the sharp decrease of the total organic phosphorus content with depth. For Methods, there is good reason to expect (0.95 probability) that some of the methods were better than others. Or, there was an indication that one or more of the differences among method means were significant. However, the F-test only accepts or rejects the homogeneity hypothesis. It gives no decisions as to which of the differences among the method means are significant in the present study.

One of the modes for testing the foregoing differences between methods more specifically is by means of a multiple range test (33), although Snedecor warns (97, p. 253)

TABLE 5 DETERMINATION OF TOTAL ORGANIC PHOSPHORUS BY VARIOUS METHODS

EXPRESSED AS P.P.M. OF OVEN-DRY SOIL

Black Solodized Solonetz (depth in inches)		A. Mehta *et al.*			B. Kaila-Virtanen			C. Hayashi-Takijima			D. Legg-Black			E. Saunders-Williams			Total P of soil
		a	b	mean	a	b	mean	a	b	mean	a	b	mean	a	b	mean	
L-H	3 - 0	540	543	542	554	573	564	420	358	389	428	432	430	590	665	627	1410
A_h	0 - 10	471	422	446	494	504	499	440	401	420	287	293	290	463	475	469	1000
B_{t_1}	11 - 24	98	68	83	80	92	86	108	108	108	65	61	63	142	123	133	470
B_{t_2}	24 - 30	32	42	37	52	70	61	33	75	54	20	17	18	56	51	53	430
C	46 - 50	70	93	81	79	79	79	23	31	27	8	10	9	24	11	18	550

TABLE 6 ANALYSIS OF VARIANCE OF TOTAL ORGANIC PHOSPHORUS DETERMINED BY VARIOUS METHODS

Source of Variation	D.F.	S.S.	M.S.	F.obs.	F.05	F.01
Replication	1	8	8			
Treatment	24	2188155	91173			
Methods	4	70456	17614	3.7	3.01	4.77
Soil	4	2040836				
M x S	16	76863	4804			
Error	24	9225	384			
Total	49	2197388				

that it is not possible to make valid comparisons of the apparent precision of the individual methods by means of this test. The five method means, being the respective means of all ten values within each method, were subjected to a multiple range test, as is shown in Table 7. The five means are arranged in descending order. The data show that the methods of Mehta et al., Kaila-Virtanen and Saunders-Williams gave similar results, at least with the five soil samples used, and that they extracted significantly greater amounts of organic phosphorus than the methods of Legg-Black and Hayashi-Takijima.

The amount of total phosphorus extracted by the five methods used to determine total organic phosphorus and expressed as a percentage of the total soil phosphorus obtained by wet digestion are presented in Table 8. A summary of the Analysis of Variance of these percentages is given in Table 9, while Table 10 assesses the significance (five per cent level) of the differences between mean percentages. The Kaila-Virtanen method extracted significantly more phosphorus than did either the Mehta et al. or the Saunders-Williams methods. In fact, the latter two methods were equal in extracting power as far as these five soil samples were concerned.

The low ignition temperature of Legg-Black's method resulted in incomplete combustion and was a likely reason for the low phosphorus values. This temperature of 240°C

TABLE 7 ASSESSMENT OF THE SIGNIFICANCE (AT FIVE PER CENT LEVEL) OF THE DIFFERENCES BETWEEN MEANS OF METHODS USED TO DETERMINE TOTAL ORGANIC PHOSPHORUS

Method	$\bar{x}$*	$\bar{x}$-162	D	$\bar{x}$-200	D	$\bar{x}$-238	D	$\bar{x}$-258	D
E. Saunders-Williams	260	98	(26)	60	(24)	22	(22)	2	(18)
B. Kaila-Virtanen	258	96	(24)	58	(22)	20	(18)		
A. Mehta *et al.*	238	76	(22)	38	(18)				
C. Hayashi-Takijima	200	38	(18)						
D. Legg-Black	162			Methods E B A C D**					

* mean of duplicate determinations of five depths (ten values)

** any two underscored by the same line are not significantly different from each other.

TABLE 8 PERCENTAGE OF TOTAL SOIL PHOSPHORUS EXTRACTED BY VARIOUS METHODS USED TO DETERMINE TOTAL ORGANIC PHOSPHORUS

Black Solodized Solonetz	A. Mehta *et al.*	B. Kaila-Virtanen	C. Hayashi-Takijima	D. Legg-Black	E. Saunders-Williams
L-H	64.5	69.1	37.7	44.7	56.4
Ah	61.2	67.3	45.3	40.9	56.0
B_{t1}	43.4	51.3	34.7	27.4	46.6
B_{t2}	52.1	62.3	45.1	28.8	45.6
C	58.2	69.1	44.4	30.2	51.6
Mean	55.9	63.8	41.4	34.4	51.2

TABLE 9 ANALYSIS OF VARIANCE OF PERCENTAGE OF TOTAL SOIL PHOSPHORUS EXTRACTED BY VARIOUS METHODS USED TO DETERMINE TOTAL ORGANIC PHOSPHORUS

Source of Variation	D.F.	S.S.	M.S.	F.obs.	F.05	F.01
Methods	4	2708	677	37.6	3.01	4.77
Soil	4	664	166			
Error	16	286	18			
Total	24	3658				

TABLE 10 ASSESSMENT OF THE SIGNIFICANCE (AT FIVE PER CENT LEVEL) OF THE DIFFERENCES BETWEEN MEANS OF PERCENTAGES OF TOTAL PHOSPHORUS EXTRACTED

Method	$\bar{x}$	$\bar{x}$-34.4	D	$\bar{x}$-41.4	D	$\bar{x}$-51.2	D	$\bar{x}$-55.9	D
B. Kaila-Virtanen	63.8	29.4	(8.2)	22.4	(7.7)	12.6	(6.9)	7.9	(5.7)
A. Mehta *et al.*	55.9	21.5	(7.7)	14.5	(6.9)	4.7	(5.7)		
E. Saunders-Williams	51.2	16.8	(6.9)	9.8	(5.7)				
C. Hayashi-Takijima	41.4	7.0	(5.7)						
D. Legg-Black	34.4			Methods B A E C D					

is, after all, an arbitrary compromise between decreased extraction of organic derived phosphorus and increased extraction of native inorganic phosphorus. The acid leaching step of the Hayashi-Takijima procedure in order to free the sample from free $CaCO_3$ and exchangeable calcium has definite merits, but it is most likely too mild in that the time of contact with the different extractants is rather short.

(b) Recovery of added organic phosphorus

The next step after studying a direct comparison of the five selected methods for determining total organic phosphorus should be a study of their behaviour when certain compounds are added to the soil samples being tested. The presence in soil of certain phosphorus bearing organic compounds is fairly well established. Anderson (6) in a comparison of methods selected five phosphate esters and added them in solution or suspension to partially dried soil. The soil with the added phosphorus was then thoroughly dried, remoistened, and dried once more. A finely ground portion was tested for total organic phosphorus. This procedure allowed the added organic phosphorus to become somewhat fixed. Another way could have been by incubating the mixed material for 24-48 hours at not too favourable temperatures, e.g. 5°-$10^{o}C$, to keep microbial growth in the lag-phase. This would allow

for a certain amount of fixation of the added phosphorus compound without much decomposition.

A different approach was taken in the present study. Organic phosphorus compounds were merely added to the dry soil after which the samples were subjected to the five methods for determining organic phosphorus. Fixation should have been slight, but this technique should at least allow an insight into the solubility and ease of hydrolysis of the compounds in the presence of soil under the different conditions of the determinations. Only two compounds, ribonucleic acid (RNA) and a calcium-magnesium salt of inositol hexaphosphoric acid (phytin), were used. It was felt that they would yield enough information regarding the five different methods for determining total organic phosphorus.

After determining the total phosphorus present in the RNA and phytin, appropriate amounts were added to samples of a Black Solodized Solonetz to supply 50, 100, 200, and 400 p.p.m. of organic phosphorus over and above that already present in the soil. The samples were then analyzed for total organic phosphorus, the results of which are recorded in Tables 11 and 12. Neither the RNA nor the phytin contained measurable quantities of orthophosphate.

TABLE 11 RECOVERY OF ORGANIC PHOSPHORUS FOLLOWING ADDITIONS OF RIBONUCLEIC ACID TO SAMPLES OF A BLACK SOLODIZED SOLONETZ (MEANS OF DUPLICATE DETERMINATIONS)

P.P.M.

Depth taken (inches)	Organic P added	Total P extracted					Inorganic P extracted					Total organic P extracted					Recovery organic P (per cent)				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
	--	820	863	404	554	839	278	299	125	239	208	542	564	279	315	631					
	50	861	895	422	604	846	343	299	124	256	212	518	596	298	348	634	--	64	38	66	6
3 - 0	100	891	922	488	638	904	368	298	125	285	209	523	624	363	353	695	--	60	84	38	64
	200	1021	981	517	725	933	423	305	125	305	207	598	676	392	420	726	28	56	57	53	48
	400	1161	1107	583	799	993	478	310	125	335	206	683	797	458	464	787	35	58	45	37	39
	--	240	262	206	187	209	212	209	159	162	159	28	53	47	25	50					
	50	239	297	210	227	239	209	210	159	192	183	30	87	51	35	56	4	68	8	20	12
24 - 30	100	267	322	275	246	270	222	207	158	211	178	45	115	117	35	92	17	62	70	10	42
	200	364	375	314	297	307	248	208	161	224	188	116	167	153	73	119	44	57	53	24	35
	400	515	511	423	448	398	332	218	161	288	190	183	293	262	160	208	39	60	54	34	40

A - method by Mehta et al.
B - method by Kaila-Virtanen
C - method by Hayashi-Takijima
D - method by Legg-Black
E - method by Saunders-Williams

TABLE 12 RECOVERY OF ORGANIC PHOSPHORUS FOLLOWING ADDITIONS OF PHYTIN TO SAMPLES OF A BLACK SOLODIZED SOLONETZ (MEANS OF DUPLICATE DETERMINATIONS)

P.P.M.

Depth taken (inches)	Organic P added	Total P extracted					Inorganic P extracted					Total organic P extracted					Recovery organic P(per cent)				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
	--	652	591	338	417	574	194	95	84	129	95	458	496	254	288	479					
	50	669	617	384	432	624	172	92	90	132	95	497	525	294	300	529	78	58	80	24	100
0 - 10	100	715	661	431	460	653	164	100	85	139	99	551	561	346	321	554	93	65	92	33	75
	200	791	758	531	512	732	168	96	81	158	110	623	662	450	354	622	83	83	98	33	72
	400	964	910	731	612	784	187	98	88	180	118	777	812	643	432	666	80	79	97	36	47
	--	240	233	231	147	217	200	169	175	129	163	40	64	56	18	54					
	50	284	261	233	148	248	199	153	177	135	151	85	108	56	13	97	90	88	-	--	86
24 - 30	100	326	291	289	177	272	197	152	178	143	160	129	139	111	34	112	89	75	55	16	58
	200	388	375	359	219	324	195	161	183	151	169	193	214	176	68	155	77	75	60	25	51
	400	529	586	550	297	420	202	157	179	170	184	327	429	371	127	236	72	91	79	27	46

A - method by Mehta et al.
B - method by Kaila-Virtanen
C - method by Hayashi-Takijima
D - method by Legg-Black
E - method by Saunders-Williams

The percentage recoveries and the ranges (values within brackets) are presented in the following tabulation:

Methods	A	B	C	D	E
RNA	21 (0-44)	61 (56-68)	51 (8-84)	35 (10-66)	36 (6-64)
Phytin	83 (72-93)	77 (58-91)	70 (0-98)	24 (0-36)	67 (46-100)

It is quite obvious that recovery was different for the five methods. The recoveries of the phytin were much higher than for RNA, because the former is rather soluble in water. But complete recovery did not occur with any method or at any level of applied organic phosphorus except in one instance.

The method of Kaila-Virtanen (B) recovered at least 50 per cent of the RNA and phytin at all levels of application. It also had the narrowest range of variation of all the methods. That is, all values were relatively closely scattered around the mean. Recovery by the Mehta _et al_. method (A) was fairly good with the phytin, but poor for the RNA. The Hayashi-Takijima method (C) was not completely comparable to the others, because the sample was leached on a filter paper with 100 ml. of one per cent HCl as a first step in its extraction procedure.

It has already been noted (Table 5) that the method of Legg-Black, with its low ignition temperature and

therefore incomplete combustion, resulted in low organic phosphorus values. The same observation was made for the recoveries of RNA and phytin. Many reports in the literature point out that, in general, ignition procedures do yield high organic phosphorus values. The recovery data, however, did not support these statements. At this stage recovery by the extraction type procedures seemed to be superior.

A closer look at the columns 'Inorganic P extracted' and 'Total P extracted' of Tables 11 and 12 is revealing. Although the behaviour of phytin was not entirely the same as RNA, the two Tables will be discussed together. Hydrolysis of the added material, or release of fixed inorganic phosphorus occurred extensively with some of the methods. The method of Mehta et al. hydrolyzed the RNA, but not the phytin. Both the methods of Kaila-Virtanen and Hayashi-Takijima do not show any sign of hydrolysis. The method of Legg-Black hydrolyzed both RNA and phytin. The results by the method of Saunders-Williams are somewhat erratic. This may not be the result of hydrolysis, because Anderson (6) did not find any hydrolysis of inositol hexaphosphate using the ignition procedure. It is possible that the clay fraction of the soils used in this study has different properties to those studied by Anderson. This may account for some of the variation.

Several phosphorus containing organic compounds have been isolated, such as inositol hexaphosphate, phospholipids, ribonucleic acid, deoxyribonucleic acid, and glucose-1-phosphate (6). These, however, account for only a small fraction of the total organic phosphorus content of soil. With such a variety of compounds, it seems improbable that one extraction procedure, even though it consisted of various steps, would extract all organic phosphorus from the soil.

Anderson (6) found that glucose-1-phosphate hydrolyzed considerably when added to soils and recovery was negligible. He found that the addition of a pre-pretreatment of 0.3N NaOH to a modified Mehta _et al_. procedure was quite effective in recovering any alkali-soluble, acid-labile phosphate esters present. It would appear that further modifications such as specific organic or inorganic extractants will be necessary to extract and study the different compounds present in the soil. Certain other organic and inorganic extractants and chelating agents will be discussed in the next section of this study. On the other hand, each extractant will aid in the formation of artifacts as a result of the relative ease of many organic reactions. Therefore, with the addition of more extraction steps certain compounds may be recovered, but others most likely will be changed.

(c) Dry combustion of organic matter

Dry combustion procedures presuppose several attributes:

- complete mineralization and extraction of the organic phosphorus is ensured,
- phosphorus is not lost by volatilization,
- the organic phosphorus present in a nonignited sample appears in the extract of the ignited sample in the form of orthophosphate (59),
- the solubility of the original inorganic phosphorus does not change as a result of the ignition (40, 59, 73),
- the ignition temperature is high enough to prevent fixation of phosphorus by hydrated iron and aluminum (73).

Certain requirements for such ignition methods are necessary, if they are to be of quantitative value. The increase in solubility of inorganic phosphorus should be kept low, and volatilization of phosphorus released from organic forms should be prevented. These can be attained by keeping the ignition temperature low. On the other hand, the fixing capacity of the soil should be low and the combustion of the organic matter should be complete, both objectives requiring high temperatures.

A series of ignitions were performed to obtain some information concerning the dry combustion procedures. Two types of peat samples were chosen in order to prevent complications from iron and aluminum phosphates in the soil. However, there will still be some inorganic phosphorus as a result of hydrolysis of organic phosphorus.

These samples were composited from the first foot of material. No mineral matter was present except for dust blown into the bogs. The one sample was light yellow-brown in colour, spongy, and not completely decomposed Sphagnum intermixed with small quantities of herbaceous material. The other sample was a dark-brown to black, fibrous, felty peat derived chiefly from Carex--and Phragmites--species.

The total phosphorus contents of the moss and sedge peats by wet digestion were 329 and 1072 p.p.m., respectively. These values are depicted in Figure 10 as straight, horizontal lines. In this Figure there are also illustrated the results of the normal dry combustion technique and several modifications of this technique as reported in the literature. The combustions are all carried out over certain temperature ranges, as shown in Figure 10. Curve 1 represents a normal ignition of the material without any additives. After the material was ignited for one hour at the particular temperature, the ash was moistened with a little water followed by the addition of 10 ml. of 6N HCl. The material was digested for 20 to 30 minutes on a waterbath, and then evaporated to dryness. The samples were heated for another 30 minutes on the water-bath in order to partially dehydrate any silica present. Dehydration was completed by placing the samples in a drying oven at 110°C for one hour. The dried salts were

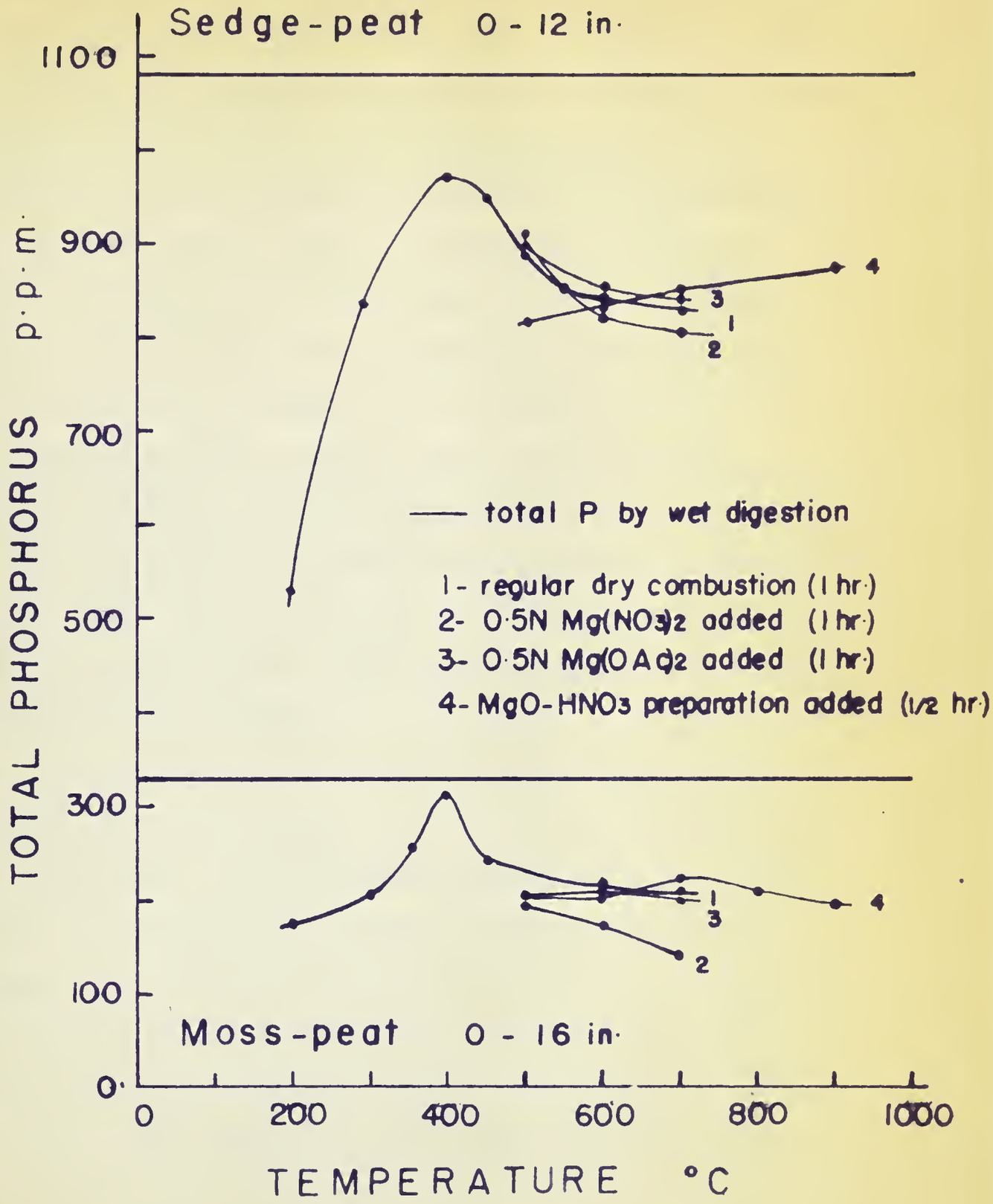

Figure 10. Total phosphorus of two peat samples determined by several dry combustion procedures at different temperatures and compared to total phosphorus by wet digestion.

moistened with 10 ml. of 6N HCl and 10 ml. of water, and heated until all soluble salts were in solution. After filtering, the solution was made up to volume and aliquots of this solution were taken for the determination of phosphorus by the phosphovanadomolybdic acid procedure. It has been reported that no phosphorus is lost by volatilization on dry-ashing when the ash is alkaline (51, p. 335). The ashing, therefore, was carried out for 1 hour in the presence of 0.5N $Mg(NO_3)_2$ or 0.5N $Mg(OAc)_2$ giving curve 2 and 3, respectively. Although $Mg(NO_3)_2$ caused some deflagration, the results with $Mg(OAc)_2$ were not much better. Finally, in curve 4, higher temperatures were used for ½ hour after adding a $MgO-HNO_3$ preparation*. The results were quite different from what was expected.

It is obvious from Figure 10 that ignitions at about 400°C produce the closest value to the one obtained by wet digestion. The difference between the two values, being 13 and 100 p.p.m. for the moss and sedge peat respectively, was recovered from the residue left on the filterpaper by a HNO_3-HClO_4 wet digestion. The data show that the temperatures used by other workers, being 600°C (54, 73), 550°C (84), and 500°-550°C (83), are disputable.

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* Method used in the Agricultural Soil and Feed Testing Laboratory of the University of Alberta, Edmonton.

(d) Dry combustion of pure compounds

The foregoing study was extended by comparing the total phosphorus values obtained by dry combustions with those obtained by HNO_3-$HClO_4$ wet digestions of certain phosphorus bearing materials for which evidence of their presence in soils has been indicated (6). The materials chosen were ribonucleic acid (RNA), deoxyribonucleic acid (DNA), the phospholipid lecithin, the calcium-magnesium salt of the hexaphosphoric acid ester of the hexavalent alicyclic alcohol meso-inositol (phytin), and dipotassium glucose-1-phosphate. The results are recorded in Table 13.

The residues left upon filtration after dry combustions at 500°, 600°, and 700°C were wet digested. The third line for each ignition temperature in Table 13 gives the total phosphorus obtained by combustion (dissolved + recovered) as a percentage of the total phosphorus obtained by wet digestion.

Anderson (6) found after adding glucose-1-phosphate to soil and determining the total organic phosphorus by an ignition procedure that the recovery of this compound was negligible. Glucose-1-phosphate hydrolyzed readily during extraction from the nonignited soil with acid solution. In the present study a black residue was obtained upon filtration of the material ignited at 500°, 600°, and 700°C. Hardly any phosphorus could be

TABLE 13 TOTAL PHOSPHORUS VALUES OF FIVE PHOSPHORUS BEARING MATERIALS COMPARING IGNITION WITH HNO_3-$HClO_4$ DIGESTION (PER CENT)

		RNA	DNA	Lecithin	Phytin	Glucose-1-Phosphate(di K)
Total P by HNO_3-$HClO_4$ digestion		8.38	9.26	3.36	14.03	8.49
500°C	Dissolved after ignition	7.75	8.70	2.02	13.74	8.40
	Recovered from residue	0.61	0.53	1.3	0.28	0.06
	Dissolved+ Recovered over digestion	99.8	99.7	98.2	99.9	99.6
600°C	Dissolved after ignition	5.77	8.42	1.49	13.01	8.34
	Recovered from residue	0.05	0.11	1.7	1.0	0.90
	Dissolved+ Recovered over digestion	69.5	92.1	94.9	99.9	99.3
700°C	Dissolved after ignition	5.28	8.06	1.99	8.70	8.29
	Recovered from residue	0.74	0.14	1.2	5.2	0.80
	Dissolved+ Recovered over digestion	71.8	88.6	94.0	99.1	98.9
800°C	Dissolved after ignition	7.25	8.55	3.11	13.5	8.36
	Ignition over digestion	86.5	92.3	92.6	96.1	98.5
900°C	Dissolved after ignition	6.91	8.78	3.02	13.4	8.44
	Ignition over digestion	82.5	94.8	89.9	95.8	99.4

recovered from this black residue when glucose-1-phosphate was the ignited material thereby supporting Anderson's findings that glucose-1-phosphate cannot be recovered by acid extraction. The residue after ignition of RNA and DNA contained, similarly, only small amounts of phosphorus.

Phytin and lecithin showed a completely different behaviour. In the case of phytin ignited at 500°, 600°, and 700°C there was 2.0, 7.2, and 37.1 per cent respectively of the total phosphorus left behind in the residue. To elucidate this behaviour of the phosphorus the following explanation may be advanced* (also see 70, pp. 648-654). As temperature increases any dihydrogen orthophosphate present is dehydrated (or aggregated) to metaphosphates, whose properties are entirely dependent upon the thermal treatment employed. With the increase in temperature these metaphosphates (cyclic or straight-chain polymers) seem to form insoluble complexes with metal ions, being in this case mainly calcium and magnesium. These complexes are difficult to convert to orthophosphates. At the temperatures of 800° and 900°C pyrophosphates are formed which can be hydrolyzed to orthophosphates by boiling with weak acid. In the case of lecithin ignited at 500°, 600°, and 700°C there was 38.1, 50.6, and 34.8 per cent respectively of the total phosphorus left behind in the residue. The carbon bondings seem to be rather strong for lecithin.

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* Dr. W. J. Wallace, Associate Professor, Department of Chemistry, University of Alberta. Personal communication.

Its resistance to oxidation was, therefore, not at all unexpected. This carbon will not be oxidized to carbon dioxide until temperatures of 750°C and up have been reached.

The data of Tables 11, 12, and 13, and Figure 10 are all quite self-explanatory. The temperatures (500° to 600°C) which are used in the ignition type determinations for total organic phosphorus result only in incomplete combustion. Snell and Snell (98, p. 496) further state that the dry ashing method is generally considered unreliable. Volatilization of the phosphorus is another cause of considerable loss. Legg-Black's temperature of 240°C may in practice be relatively satisfactory, however, after the foregoing results it is difficult to visualize it as a proper approach towards a better understanding of the organic phosphorus status of soils. All the foregoing evidence made it necessary to turn to extraction procedures for estimating soil organic phosphorus.

(e) Summary

A brief summary of this section is given to emphasize some of the important findings. From the work done on the Black Solodized Solonetz using the two ignition methods, which according to the literature usually gave high organic phosphorus values, the method of Legg-Black

was found to be unsatisfactory. Saunders-Williams' method, although not significantly better than two extraction type procedures (Table 7), did permit a certain amount of hydrolysis. High total organic phosphorus values most likely resulted from inorganic phosphorus coming into solution as a result of ignition. From the work done on the peat samples and, particularly, the pure compounds it became quite clear that the temperatures which have been used in the ignition type determinations resulted only in incomplete combustion. Volatilization of the phosphorus was another cause of considerable loss at least for certain compounds studied.

For the extraction type procedures Kaila-Virtanen's method with the mild acid extraction seemed to be the best approach. However, as long as one does not know more about the nature of the compounds and complexes involved, it is impossible to predict and prove which extraction method will be the best for any given soil. In addition to different forms of organic phosphorus other factors may complicate the pursuit made in order to obtain a better knowledge with regard to the organically bound phosphorus present in soil. Anderson (6) found, for example, increased stability of inositol hexaphosphate and β-glycerophosphate in calcareous soils as compared with acid soils, but thought it unlikely to be a function of soil pH or carbonate content. The present methods are, therefore, very empirical indeed.

(C) Possible Modifications of the Kaila-Virtanen Extraction for Determining Total Organic Phosphorus

It was shown by the addition of known compounds that all methods studied in the previous section were very empirical indeed. The third objective of this investigation was to ascertain a workable modification of one or more of these methods particularly with the aid of the latest findings in the use of organic matter extractions.

The accuracy of any method determining organic phosphorus in soils is difficult to prove, because there is not a satisfactory absolute standard with which comparisons can be made. Recoveries are helpful, but not ideal because it is difficult to determine what changes take place in compounds as a result of decomposition or fixation when they are added to the soil. The method which extracts the largest amount of organic phosphorus may be considered to be the most accurate, although nothing can be concluded regarding its absolute accuracy (67). The procedure of Kaila-Virtanen was chosen as the method against which any modifications were to be compared. This decision was based on the following findings:

1. Evidence against dry combustion procedures was quite conclusive.

2. Although not consistent the acid pretreatment of the Kaila-Virtanen procedure seemed to remove more completely $CaCO_3$ and exchangeable calcium from the soil than the other extraction methods tried.

3. The acid pretreatment of the Kaila-Virtanen procedure is rather mild.

4. With regard to percentages of total soil phosphorus extracted, the Kaila-Virtanen procedure extracted significantly more phosphorus than did either the Mehta et al. or the Saunders-Williams methods. All three methods were equal as far as determining total organic phosphorus content was concerned.

5. The method of Kaila-Virtanen recovered at least 50 per cent of added RNA and phytin, more than any of several other methods tried.

6. No sign of hydrolysis of added RNA and phytin was found.

Any modification that produced lower total organic phosphorus values was discarded. This does not mean, however, that such a modification may not be useful for studying the nature and identity of certain compounds extracted. But this would be qualitative, while the present investigation was towards quantitative total extractions.

(a) First modification of Kaila-Virtanen's procedure

The Kaila-Virtanen method commences with a 4N H_2SO_4 extraction for 18 hours at room temperature after which the samples are treated at room temperature with 0.5N NaOH at 90°C for four hours. As a first modification, the acid extraction was performed on a shaker for four hours. If the results did not differ from the 18 hours extraction it would definitely be a time saving feature. It is evident from Table 14 that the four hours acid extraction on a shaker is permissible.

TABLE 14 FIRST MODIFICATION OF KAILA-VIRTANEN'S PROCEDURE FOR EXTRACTING TOTAL ORGANIC PHOSPHORUS (P.P.M.)

	Method of Kaila-Virtanen (18 hrs. with 4N H_2SO_4)			4 hrs. shaking with 4N H_2SO_4, rest as Kaila-Virtanen		
	Total P extracted	Inorganic P extracted	Organic P	Total P extracted	Inorganic P extracted	Organic P
Cooking Lake Loam L-H						
Combined analysis of extracts	561	167	394	555	155	400
Separate analysis of extracts	585	194	391	613	176	437
Cooking Lake Loam Bt						
Combined analysis of extracts	87	6	81	98	9	89
Separate analysis of extracts	106	22	84	109	24	85

Another feature of the data in Table 14 is that under the conditions of this study it did not appear to make any difference whether the extractions were analyzed separately or together. The purpose of analyzing the acid and alkaline extracts separately was to eliminate the possible adsorption of inorganic phosphorus by the precipitated humic acids of the alkali extracts when they were in contact with the acid extracts. Kaila and Virtanen (54) did not find it necessary to make these separate determinations and the foregoing results seem to substantiate this finding. However, the extracts *per se* were kept separate and only the aliquots taken from these extracts were mixed. This was done in order to prevent any error as a result of flocculation of humic acid when the acid and alkaline extracts were mixed before aliquots were taken, thereby resulting in a non-homogeneous solution.

(b) Interferences with molybdophosphoric blue colour

It was pointed out in the literature review that recently many organic agents have been used in an effort to obtain more complete extraction of organic matter. Further, extraction with organic solvents should precede extraction with NaOH so as to obtain low-molecular, unstable, humic substances (85). At first some of the organic solvents recommended in the literature were

used, however it soon became clear that many of these solvents greatly interfered with the molybdophosphoric blue colour estimation. It was also found, with the aid of standards (KH_2PO_4), that it actually made a difference if the determinations were made in an HCl--or in a H_2SO_4--system. A similar observation was made by Robertson (80), who showed that a far more intense colour was obtained with H_2SO_4 and pointed out that it was largely because of this high intensity with its resultant sensitivity that most workers preferred to use this acid. Further, if either HCl or H_2SO_4 was used in any phase of the extraction procedure one should be consistent by using ammonium molybdate reagent prepared with either HCl or H_2SO_4 (Also see 46).

Table 15 illustrates some of the points mentioned in the previous paragraph. All solvents mentioned are completely miscible with water except acetic anhydride (13.6 gm./100 ml., cold). Ten milliliters of 1:1 solutions (five milliliters of acetic anhydride) were added to appropriate aliquots of KH_2PO_4 standards. For the HCl-system the solutions were neutralized with 1:1 NH_4OH and 0.5N HCl using p-nitrophenol as indicator. Five milliliters of ammonium molybdate reagent prepared with HCl (67) were added and the solutions made up to 100 ml. For the H_2SO_4-system the solutions were neutralized with 1:1 NH_4OH and 2N H_2SO_4 using p-nitrophenol as

TABLE 15 PER CENT TRANSMITTANCY READINGS OF MOLYBDOPHOSPHORIC BLUE COLOUR IN EITHER HCl- or H_2SO_4-SYSTEMS USING A NUMBER OF ORGANIC EXTRACTANTS

P.p.m. of P as KH_2PO_4	0	0.3	0.5	0.7
HCl-system				
Standard curve	100	58.9	41.7	29.5
Dioxan	100	66.8	50.8	38.6
Acetone	100	64.8	48.3	36.5
Ethanol	100	61.1	45.5	34.2
Form-dimethylamide	100	69.0	71.9	77.3
Pyridine	All solutions, including the blank, turned to a dark blue.			
Acetic Anhydride				
Formic acid				
Lactic acid	No colour development.			
H_2SO_4-system				
Standard curve	100	54.2	36.9	25.0
Dioxan	100	59.2	40.0	27.2
Acetone	100	55.9	37.8	25.0
Ethanol	100	56.8	37.8	24.9
Form-dimethylamide	100	54.9	61.5	70.1
Pyridine	All solutions, including the blank, turned to a dark blue.			
Acetic Anhydride				
Formic acid				
Lactic acid	No colour development.			

indicator. Five milliliters of 2½ per cent sulfomolybdic acid solution (51, p. 142) were added and the solutions made up to 100 ml. $SnCl_2$, as suggested by Mehta et al. (67), was used as a reducing agent for both systems.

Lactic acid prevented blue colour development. Pyridine, acetic anhydride, and formic acid, on the other hand, turned the solutions into a dark-blue colour including the blank. Dimethyl-formamide caused increased turbidity with increased KH_2PO_4 concentrations even before the $SnCl_2$ was added to develop the colour. This suspension must also remove some phosphorus from solution, because with increased concentrations decreased transmission was observed. Dioxan, acetone, and ethanol developed normal colour patterns. The transmission obtained when ethanol was present in a H_2SO_4-system came closest to the readings of the standard curve, followed by acetone and dioxan in the same system. Scheffer et al. (85) found dioxan to be a good organic matter extractant, while acetone and ethanol were particularly suited for extracting low-molecular, unstable, humic substances.

Aqueous solutions of the salt NaF and of the metal chelating organic compounds 8-hydroxy quinoline, cupferron, and acetyl acetone have been used in the past with some success. These compounds had to be checked first for interference with the molybdenum blue procedure. For this purpose 1:1 solutions of dioxan, acetone, and

ethanol were made up containing either 0.2M acetyl acetone, 0.05M cupferron, 2½ per cent oxine, or 0.5M NaF. The results for per cent transmission are tabulated in Table 16. Oxine can definitely be eliminated. Acetyl acetone, particularly with acetone, did not suppress the molybdophosphoric blue colour to a great extent. Martin and Reeve (63) found acetyl acetone to be the most useful metal chelating reagent of any tested. On the other hand, Choudhri and Stevenson (29) found neutral pyrophosphate to be most effective in removing organic matter from surface soils, while cupferron was more effective than acetyl acetone.

Fluoric acid was the next compound introduced into the trials to determine its interference with the molybdophosphoric blue colour. To different phosphorus standards, 10 ml. 1:1 dioxan, acetone, or ethanol containing 0.2M acetyl acetone, 10 ml. 0.3N NaOH, either 10 ml. 4N H_2SO_4 or 10 ml. of a solution of 4N H_2SO_4-2N HF, and 20 ml. of 0.5N NaOH were added, after which the solutions were made up to 100 ml. $SnCl_2$, as suggested by Mehta _et al_. (67), was used as a reducing agent for both systems.

It is evident from Table 17 that the fluoride-ion produced a negative interference with the molybdophosphoric blue reaction. Kurtz (58) pointed out that the interference

TABLE 16 PER CENT TRANSMITTANCY READINGS OF MOLYBDOPHOSPHORIC BLUE COLOUR IN A H_2SO_4-SYSTEM OF AQUEOUS SOLUTIONS CONTAINING ONE OF A NUMBER OF METAL COMPLEXING COMPOUNDS

P.p.m. of P as KH_2PO_4	0	0.3	0.5	0.7
Standard curve	100	54.2	36.9	25.0
Dioxan				
0.5M sod.fluoride	100	63.1	44.9	32.1
0.2M acetyl acetone	100	59.8	39.9	26.9
0.05M cupferron	100	95.6	92.9	84.1
2½ % oxine	Highly turbid, hardly any colour.			
Acetone				
0.5M sod.fluoride	100	63.0	45.1	32.1
0.2M acetyl acetone	100	55.4	37.4	27.1
0.05M cupferron	100	59.4	47.9	38.2
2½ % oxine	Highly turbid, hardly any colour.			
Ethanol				
0.5M sod.fluoride	100	75.8	67.2	62.2
0.2M acetyl acetone	100	59.5	37.2	27.1
0.05M cupferron	100	98.5	97.1	96.5
2½ % oxine	Highly turbid, hardly any colour.			

TABLE 17 PER CENT TRANSMITTANCY READINGS OF MOLYBDOPHOSPHORIC BLUE COLOUR IN A H_2SO_4-SYSTEM COMPARING DIFFERENT STRENGTHS OF HF WITHOUT AND WITH H_3BO_3

P.p.m. of P as KH_2PO_4	0	0.3	0.5	0.7
Standard curve	100	54.2	36.9	25.0
Dioxan + 0.2M acetyl acetone				
4N H_2SO_4	98.5	63.5	45.5	34.5
4N H_2SO_4 + 2N HF	no colour development			
4N H_2SO_4 + 2N HF + 0.8M H_3BO_3	100	58.8	38.7	26.7
4N H_2SO_4 + 1N HF + 0.8M H_3BO_3	100	59.5	40.9	27.2
Acetone + 0.2M acetyl acetone				
4N H_2SO_4	100	56.1	37.8	24.8
4N H_2SO_4 + 2N HF	no colour development			
4N H_2SO_4 + 2N HF + 0.8M H_3BO_3	100	59.8	39.1	28.8
4N H_2SO_4 + 1N HF + 0.8M H_3BO_3	100	61.9	40.9	29.2
Ethanol + 0.2M acetyl acetone				
4N H_2SO_4	100	56.5	36.6	24.5
4N H_2SO_4 + 2N HF	no colour development			
4N H_2SO_4 + 2N HF + 0.8M H_3BO_3	100	59.7	41.9	31.5
4N H_2SO_4 + 1N HF + 0.8M H_3BO_3	100	61.5	42.8	32.3

of the fluoride-ion, if present in greater amounts than about five parts per million, can be eliminated by the addition of boric acid, as follows $4F^- + H_3BO_3 + 3H^+ \rightleftharpoons (BF_4)^- + 3H_2O$. The reaction is reversible, but hydrolysis is slow at room temperature (70, p. 761). Neither the boric acid ions nor the fluoroborate-ions interfere with the molybdophosphoric blue reaction. In the case of total phosphorus analyses, where the solution is digested with $HClO_4$, the fluoride-ion is automatically removed by evaporation. Ten milliliters of 4N H_2SO_4-2N HF and five milliliters of 4N H_2SO_4-1N HF were added, together with 25 ml. or 15 ml. 0.8M H_3BO_3, respectively. The results are also presented in Table 17.

To summarize the data of Tables 15, 16, and 17, it follows that from the standpoint of least interference with the molybdophosphoric blue colour:

1.-a H_2SO_4-system is preferred,

2.-ethanol and acetone perform best of the extractants tested,

3.-acetyl acetone in acetone is better than in ethanol, although both show some negative interference,

4.-ethanol and acetone plus 0.2M acetyl acetone and 4N H_2SO_4 as acid extractant warrant investigation with actual soil samples,

5.-if HF is used 2N HF and 0.8M H_3BO_3 both with ethanol or acetone also warrant investigation with actual soil samples.

(c) Second modification of the Kaila-Virtanen procedure

As recommended in the summary of the previous section, 1:1 acetone and 1:1 ethanol without and with 0.2M acetyl acetone and either with 4N H_2SO_4 or 4N H_2SO_4-2N HF were tried on two horizons of a Grey Wooded soil, namely a Cooking Lake loam L-H and Bt. The results are recorded in Table 18. Ten milliliters of 0.8M H_3BO_3 were added to the tubes in which the colour was developed as described by Mehta et al. (67). It was obvious that a marked difference existed between the two types of soils used. The L-H horizon is an organic horizon. Consequently, there was little or no silica present to react with at least some of the HF, thereby resulting in still enough HF present even after the addition of H_3BO_3 to interfere with the molybdophosphoric blue colour. With the samples from the Bt horizon, five milliliters of 0.8M H_3BO_3 sufficed. The tetrafluoride of silicon has its boiling point at minus 95.7°C (70, p. 679) with the result that in horizons with siliceous material most of the fluoride will have volatilized.

From the results in Table 18, the following procedures were established. For soil samples high in organic matter one gram of soil (100 mesh) is treated with 25 ml. 1:1 acetone in a centrifuge tube graduated at 60 ml., which is stoppered, and shaken for four hours. It is centrifuged

TABLE 18 DETERMINATION OF TOTAL ORGANIC PHOSPHORUS BY VARIOUS COMBINATIONS OF EXTRACTING AGENTS AND EXPRESSED AS P.P.M. OF OVEN-DRY SOIL

	1:1 acetone		1:1 ethanol		1:1 acetone + 0.2M acetyl acetone		1:1 ethanol + 0.2M acetyl acetone	
	4N H_2SO_4	4N H_2SO_4-2N HF	4N H_2SO_4	4N H_2SO_4-2N HF	4N H_2SO_4	4N H_2SO_4-2N HF	4N H_2SO_4	4N H_2SO_4-2N HF
Cooking Lake loam								
L - H	405	216	404	314	370	155	365	229
Bt	54	82	51	73	44	85	52	75

and the supernatant decanted into a 100 ml. volumetric flask. Sixty milliliters 0.3N NaOH are added to the soil sample, which is again shaken for four hours. It is centrifuged and the supernatant decanted into the same 100 ml. volumetric flask, which is then made up to the mark and thoroughly mixed.

To determine the inorganic phosphorus, 20 ml. are transferred on the same day to a test tube graduated at 35 ml. Add one drop of 0.5 per cent aqueous solution of p-nitrophenol, and add 2N H_2SO_4 dropwise until the colour of the indicator is discharged. Dilute to 35 ml. with water, add five milliliters of 2½ per cent sulfomolybdic acid solution (51, p. 142), and mix thoroughly. After centrifuging the solutions, add three drops of $SnCl_2$ solution (67) and immediately mix again. The presence of a brown colour requires a correction for the inherent colour of the solution by a technique proposed by Dyer and Wrenshall (34) and outlined in detail by Mehta et al. (67). A blank is not necessary in this case, but it requires a separate standard curve. This colour correction is made before addition of the $SnCl_2$. Ten minutes after addition of the $SnCl_2$ measure the percentage transmittance of the sample at a wavelength of 660 mu.

In the meantime 25 ml. 4N H_2SO_4 are added to the soil sample, which is once more shaken for four hours. It is centrifuged and the supernatant decanted into a 50 ml.

volumetric flask and diluted to volume. Next, the soil residue is treated overnight with 60 ml. 0.5N NaOH. After centrifuging the soil suspension and decanting the supernatant into a 200 ml. volumetric flask, the residue is treated with 60 ml. of 0.5N NaOH in an oven at 90°C for four hours. The supernatant is added to the previous 0.5N NaOH extract and the 200 ml. volumetric flask made up to volume.

To determine the inorganic phosphorus present in the acid, second and third alkali extracts, five milliliters of the acid and 20 ml. of the combined second and third alkali extracts are transferred by pipette to a 50 ml. volumetric flask. Five milliliters of 1N H_2SO_4 are added to ensure the precipitation of the humic acids and the flask brought up to volume. The well-mixed suspension is then centrifuged, and 20 ml. are transferred to a test tube graduated at 35 ml. Add one drop of 0.5 per cent aqueous solution of p-nitrophenol, neutralize the solution with 1:1 NH_4OH, and add 2N H_2SO_4 dropwise until the colour of the indicator is discharged. Dilute to 35 ml. with water, add five milliliters of 2½ per cent sulfomolybdic acid solution, and mix thoroughly. The presence of a brown colour requires a correction. Add three drops of $SnCl_2$ solution, and immediately mix again. Ten minutes after addition of the $SnCl_2$ measure the percentage transmittance of the sample at a wavelength of 660 mu.

The destruction of organic matter for the determination of total phosphorus extracted is performed by a wet digestion procedure. In a 100 ml. beaker place 10 ml. of the organic-first alkali extract, five milliliters of the acid extract, and 20 ml. of the combined second and third alkali extracts. Add two milliliters of 72 per cent $HClO_4$, and heat on a hot plate until fumes of $HClO_4$ appear, whereupon the beaker is immediately covered with a watch glass to reduce further loss of the acid. The beaker is removed from the hot plate when the colour of the solution no longer changes and the condensing line of the $HClO_4$ is about halfway up the side. Do not heat to dryness. When the beaker has cooled, transfer the contents quantitatively to a 50 ml. volumetric flask. Dilute the solution to volume with water, and mix thoroughly. Pipette an aliquot (10-20 ml.) into a test tube graduated at 35 ml. and proceed as described previously. Subject a blank to the same procedure simultaneously with the samples.

The following extracts are finally obtained:

1. a. 25 ml. 1:1 acetone extract
   b. 60 ml. 0.3N NaOH extract
   } into 100 ml. flask.
2. 25 ml. 4N H_2SO_4 extract into 50 ml. flask.
3. a. 60 ml. 0.5N NaOH extract
   b. 60 ml. 0.5N NaOH extract (at 90°C)
   } into 200 ml. flask.

Inorganic P found after $HClO_4$ treatment of combined aliquots − (inorganic P in 1. + inorganic P in (2. + 3.)) = Total Organic P Extracted

For soil samples low in organic matter the whole procedure is similar to that described for samples high in organic matter except for the following modifications. For the organic extraction use 25 ml. of 1:1 acetone containing 0.2M acetyl acetone. For the acid extraction use 25 ml. 4N H_2SO_4-2N HF. This requires the addition of five milliliters of 0.8M H_3BO_3 just before the neutralized combined aliquots of the acid, second, and third alkali extracts in the test tube graduated at 35 ml. is diluted to 35 ml. with water.

It was reasoned in an earlier section that filtration was better than centrifugation. In the above procedure, however, everything was centrifuged. Due to the use of several consecutive extractions the amount of filterpaper added to the soil samples would become prohibitive.

Table 19 gives the results of the determination of total organic phosphorus by the Mehta _et al_., Kaila-Virtanen, and the modified Kaila-Virtanen procedures. Tables 20 and 21 show the Analysis of Variance and assessment of the significance (at five per cent level) of the difference between the means of the data recorded in Table 19. There was statistically no difference between the modified Kaila-Virtanen and the Mehta _et al_. procedures. In spite of all efforts not much progress has been made. On the other hand, it looked as though for the B-horizon, the modified Kaila-Virtanen procedure had an advantage,

TABLE 19 DETERMINATION OF TOTAL ORGANIC PHOSPHORUS BY VARIOUS METHODS EXPRESSED AS P.P.M. OF OVEN-DRY SOIL

	A. Mehta *et al.*	B. Kaila-Virtanen	C. Modified Kaila-Virtanen
Thin Black			
Ah	615	548	612
Bm	56	76	98
Black			
Ah	738	674	737
Bm	195	170	184
Dark Grey			
L-H	542	459	546
Btj	46	29	80
Grey Wooded			
L-H	392	366	389
Bt	54	70	95
Mean	330	299	343

TABLE 20 ANALYSIS OF VARIANCE OF TOTAL ORGANIC PHOSPHORUS DETERMINED BY VARIOUS METHODS

Source of Variation	DF	SS	MS	F obs.	F 0.05	F 0.01
Methods	2	8039	4020	9.2	3.74	6.51
Soils	7	1451792	207399	472.4		
Error	14	6143	439			
Total	23	1465974				

TABLE 21 ASSESSMENT OF THE SIGNIFICANCE (AT FIVE PER CENT LEVEL) OF THE DIFFERENCES BETWEEN MEANS OF METHODS USED TO DETERMINE TOTAL ORGANIC PHOSPHORUS

Method	$\bar{x}$	$\bar{x}$ - 299	D	$\bar{x}$ - 330	D
C. Modified Kaila-Virtanen	343	44	(27)	13	(22)
A. Mehta *et al.*	330	31	(22)		
B. Kaila-Virtanen	299				C A B

but this was not born out statistically for the samples tested. The results for the Bm-horizon of the Black soil counteracted the results of the other three profiles.

Recoveries of organic phosphorus added to samples of Cooking Lake loam L-H and Bt horizons, and recorded in Table 22, were rather different from those presented in Tables 11 and 12. The modified Kaila-Virtanen procedure did not greatly improve the recovery of phytin, but it did substantially improve the recovery of RNA. For glucose-1-phosphate the method by Anderson (6) may be the most desirable one. With the modified Kaila-Virtanen procedure some hydrolysis took place with regard to the glucose-1-phosphate in both the L-H and Bt samples. The same was true for the phytin with the L-H sample.

It should be noted for the standards reported in Table 17 that some inhibition of the full molybdophosphoric blue colour occurred. As a result the values in Table 19 and 22 are lower than they should be. From the standpoint of obtaining an approximate idea of the total organic phosphorus present in a soil, it is probably faster to use the first modification of the Kaila-Virtanen procedure, i.e. four hours shaking with 4N H_2SO_4 followed by alkali extractions as outlined by Kaila and Virtanen (54). However, in the opinion of the author it is not possible to devise a single method to determine or extract all organic phosphorus present in the soil, even if several

TABLE 22 RECOVERY BY A MODIFIED KAILA-VIRTANEN PROCEDURE OF ORGANIC PHOSPHORUS FOLLOWING ADDITIONS OF VARIOUS PHOSPHORUS BEARING MATERIALS TO SAMPLES OF A GREY WOODED SOIL (MEANS OF DUPLICATE DETERMINATIONS)

	P.P.M.			
	Total P extracted	Inorganic P extracted	Total organic P extracted	Recovery organic P (per cent)
Cooking Lake loam L-H	428	39	389	
RNA	560	75	485	96
DNA	569	80	489	100
Lecithin	574	89	485	96
Phytin	610	133	468	79
Glucose-1-phosphate	585	130	455	66
Cooking Lake loam Bt	142	27	95	
RNA	199	20	179	84
DNA	199	24	175	80
Lecithin	217	21	196	101
Phytin	204	20	184	89
Glucose-1-phosphate	212	55	157	62

successive extractions are used. There are too many different forms of organic phosphorus present in the soil, many of which are still unknown. It has also been found that almost one-half of the total organic phosphorus extracted by alkali was associated with the acid-insoluble precipitate (1). Further, humic acid contains aromatic structures (123). Therefore, a better appreciation of the bonds between humic acid and phosphorus is also necessary. For a better understanding of the organic phosphorus status one should firstly determine qualitatively the forms present in the soil, and secondly devise procedures which will determine quantitatively each form. Some of the organic extractants used may prove to be very useful in this approach. Work done by Anderson (3, 4, 5, 7), MacLean (60), and Thomas and Lynch (101), for example, are definite steps in this direction.

SUMMARY AND CONCLUSIONS

The knowledge with regard to organic phosphorus, which is the phosphorus that is present in the soil as a result of biological fixation of inorganic phosphorus by plants, animals, and microorganisms, is very inadequate. The different methods available for the determination of soil organic phosphorus are open to criticism. Correspondingly, the organic phosphorus content in Alberta soils has not received much attention in the past.

The first objective of the present study was to evaluate two extraction type procedures, the Kaila and Virtanen method (54) and the Mehta et al. method (67), because these two methods have been disputed in the literature as to which will yield the greater amount of organic phosphorus. Subsequently, the study was expanded to determine whether a relationship existed between organic phosphorus and other soil properties including pH, total phosphorus, organic carbon, organic nitrogen, and non-sulphate sulphur. The second objective was to evaluate an additional extraction type procedure (49) and two ignition type procedures (59, 84) which have been used by various workers for determining the total organic phosphorus content of soils. The third objective was to ascertain a workable modification of one or more of these methods particularly with the aid of the latest findings in the use of organic matter extractions.

I. Six soils, typical of the different major soil zones in Alberta, were sampled in duplicate in the field. Two extraction type procedures, one having a strong acid (67) and the other a weak acid (54) pretreatment, were used to determine total organic phosphorus. For the soils under study, both methods were similar, although in some cases the Mehta et al. procedure did not completely remove the $CaCO_3$ and exchangeable calcium. Total organic phosphorus obtained with both methods decreased with increased depth. The decrease in the organic phosphorus content of the Brown and Dark Brown soil series with increased depth was much more gradual than for the other soil series tested. It was postulated that fewer forms of organic phosphorus were present in the Brown and Dark Brown surface horizons than in the Thin Black, Black, Dark Grey, and Grey Wooded soils. The organic phosphorus content in the upper horizon increased from the Brown to the Black, and decreased again with the Dark Grey, being respectively 172, 247, 479, 625, and 415 p.p.m. In terms of per cent organic phosphorus of total phosphorus these figures were 32, 37, 43, 50, and 39 per cent, respectively. This trend was true for both methods tested.

A high correlation existed between organic and total phosphorus, especially in the upper horizons all of which were somewhat acidic. A close association existed between organic carbon, organic nitrogen, and non-sulphate sulphur

for samples from all soil series. Non-sulphate sulphur, particularly, was highly correlated with both organic carbon and nitrogen indicating that most of the non-sulphate sulphur was present in organic forms. The relationship of organic phosphorus with non-sulphate sulphur was remarkably high, particularly in samples with a pH less than 7.0 and containing less than 500 p.p.m. of non-sulphate sulphur. In view of the high correlations it was postulated that organic nitrogen, organic carbon, organic phosphorus, and non-sulphate sulphur were all integral parts of organic matter, at least for the different soils studied. The fact that probably small amounts of inositol hexaphosphates were present should be further evidence of this relationship.

II. The foregoing data gave some indication of the behaviour of the two extraction type methods for determining total organic phosphorus. In the second part of this investigation five methods, the two previously mentioned extraction procedures, an additional extraction procedure (49), and two ignition procedures (59, 84), were selected for further study.

Ovendry samples of five horizons of a Black Solodized Solonetz were analyzed using the five methods. The data showed that the methods of Mehta _et al_. (67), Kaila and Virtanen (54), and Saunders and Williams (84) gave similar results, at least with the five soil samples used. Saunders-

Williams' method did permit a certain amount of hydrolysis. High total organic phosphorus values for this method most likely resulted from inorganic phosphorus brought into solution as a result of ignition. On the basis of percentages of total soil phosphorus extracted, the Kaila-Virtanen method extracted significantly more phosphorus than any of the other methods. The two ignition procedures appeared to be unsatisfactory as a result of incomplete combustion or of increased solubility of the original inorganic phosphorus. However, by the addition of known compounds it was shown that all methods were very empirical indeed.

To study the more rapid and simple dry combustion procedures somewhat further, first two peat samples, and then pure compounds, RNA, DNA, lecithin, phytin, and glucose-1-phosphate, were analyzed. It became quite apparent that the temperatures which have been used by other workers in the ignition type determinations gave only incomplete combustion. Volatilization of the phosphorus was another cause of considerable loss at least for certain compounds analyzed.

III. Finally, an attempt was made to improve on the Kaila-Virtanen procedure. From the standpoint of obtaining an approximation of the total organic phosphorus present in soil, a slight modification of the Kaila-Virtanen procedure seemed useful. It entailed four hours shaking with

4N H_2SO_4, instead of 18 hours of standing, and followed by alkaline extractions as outlined by Kaila and Virtanen (54). A more extended modification allowed for fairly good recoveries of added compounds and particularly for some samples of B horizons a big improvement was observed. For samples high in organic matter, 1:1 acetone, 0.3N NaOH, 4N H_2SO_4, 0.5N NaOH, and 0.5N NaOH at 90°C were used as successive extractants. For samples low in organic matter, the procedure called for 1:1 acetone containing 0.2M acetyl acetone, 0.3N NaOH, 4N H_2SO_4-2N HF, 0.5N NaOH, and 0.5N NaOH at 90°C.

However, it was reasoned that no single method can possibly estimate all the phosphate esters, or break all the humic acid-phosphate bonds present in different horizons of various soils. New approaches are needed, such as differential thermal-, infrared-, electrophoretic-, and chromatographic analyses. It is no longer a question of a few, simple, rapid extractions at relatively low cost, but painstaking, expensive, time consuming, step by step analyses will have to be carried out.

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-Appendix-

Appendix, item i.

Translation of quote given in Introduction.

The copper apocrenate, which has been obtained by a previous described method, can be used for further analysis. Other substances, brought into solution by potassium hydroxide and precipitated on the addition of copper acetate, due to the formation of insoluble compounds with copper oxyde, are chiefly silici and phosphoric acids. These compounds, however, don't interfere with the further analysis of copper apocrenate.

Appendix, item ii.

Legal Locations.

Soil type	Date sampled	Legal location
Soils of Table 3a		
1. Shallow Chin loam	September 24, 1959	NE 10- 8-14-W4
2. Lethbridge loam	September 24, 1959	NE 11- 9-20-W4
3. Airdrie loam	July 21, 1959	NE 15-28-29-W4
4. Antler loam	July 21, 1959	NE 24-32- 3-W5
5. Falun loam	November 2, 1959	SW 14-52-23-W4
6. Cooking Lake loam	July 6, 1959	NW 7-51-21-W4
Soils of Table 3b		
1a. Shallow Chin loam	August 28, 1945	NW 27-13-14-W4
2a. Lethbridge sandy loam	August 10, 1940	NW 33-22-23-W4
3a. Airdrie loam	August 15, 1941	NE 24-30-28-W4
4a. Antler loam	August 16, 1957	SE 2-36-28-W4
5a. Falun loam	June 6, 1960	SE 30-51-23-W4
6a. Cooking Lake loam	May 26, 1958	NE 12-53-21-W4
7. Black Solodized Solonetz	September 28, 1956	SW 6-47-19-W4
8. Sedge peat	July 14, 1959	SE 28-71- 8-W6
9. Moss peat	July 14, 1959	SE 14-74-12-W6